

RESEARCH ARTICLE

Vitamin D Deficiency in Early Pregnancy

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Abstract

Objective

Vitamin D deficiency is a common problem in reproductive-aged women in the United States. The effect of vitamin D deficiency in pregnancy is unknown, but has been associated with adverse pregnancy outcomes. The objective of this study was to analyze the relationship between vitamin D deficiency in the first trimester and subsequent clinical outcomes.

Study Design

This is a retrospective cohort study. Plasma was collected in the first trimester from 310 nulliparous women with singleton gestations without significant medical problems. Competitive enzymatic vitamin D assays were performed on banked plasma specimens and pregnancy outcomes were collected after delivery. Logistic regression was performed on patients stratified by plasma vitamin D concentration and the following combined clinical outcomes: preeclampsia, preterm delivery, intrauterine growth restriction, gestational diabetes, and spontaneous abortion.

Results

Vitamin D concentrations were obtained from 235 patients (mean age 24.3 years, range 18-40 years). Seventy percent of our study population was vitamin D insufficient with a serum concentration less than 30 ng/mL (mean serum concentration 27.6 ng/mL, range 13-71.6 ng/mL). Logistic regression was performed adjusting for age, race, body mass index, tobacco use, and time of year. Adverse pregnancy outcomes included preeclampsia, growth restriction, preterm delivery, gestational diabetes, and spontaneous abortion. There was no association between vitamin D deficiency and composite adverse pregnancy outcomes with an adjusted odds ratio of 1.01 (p value 0.738, 95% confidence intervals 0.961-1.057).

Conclusion

Vitamin D deficiency did not associate with adverse pregnancy outcomes in this study population. However, the high percentage of affected individuals highlights the prevalence of vitamin D deficiency in young, reproductive-aged women.



OPEN ACCESS

Citation: Flood-Nichols SK, Tinnemore D, Huang RR, Napolitano P, Ippolito DL (2015) Vitamin D Deficiency in Early Pregnancy. PLoS ONE 10(4): e0123763. doi:10.1371/journal.pone.0123763

Academic Editor: Ana Claudia Zenclussen, Medical Faculty, Otto-von-Guericke University Magdeburg, GERMANY

Received: October 15, 2014

Accepted: February 23, 2015

Published: April 21, 2015

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Data Availability Statement: All relevant data are within the paper.

Funding: The authors received no specific funding for this work.

Competing Interests: The authors have declared that no competing interests exist.

Introduction

Vitamin D deficiency is a common problem in reproductive aged women in industrialized countries and its prevalence may be increasing [1]. The etiology for this increase is likely multifactorial, but due in part to low dietary intake of vitamin D and limited exposure to sunlight. Certain high risk groups for vitamin D deficiency in pregnancy have been identified, including vegetarians, women with limited sun exposure (e.g., those who live in cold climates, northern latitudes, or wear sun and winter protective clothing), and ethnic minorities, especially those with darker skin [2]. Vitamin D deficiency is also more common among heavier women than leaner individuals [3]. During pregnancy, severe maternal vitamin D deficiency, as defined as serum 25-hydroxyvitamin D (25-OH vitamin D) concentrations less than 5 ng/mL, has been associated with disordered skeletal homeostasis, congenital rickets, and fractures of the newborn [4]. However, the effect of less severe vitamin D deficiency and insufficiency on maternal and fetal outcomes during pregnancy is less clear. Recent observational and randomized control trials have attempted to investigate this issue, but there remains limited guidance on the management of vitamin D deficiency during pregnancy.

Vitamin D deficiency and insufficiency have been associated with a variety of adverse maternal and fetal outcomes, ranging from preeclampsia, gestational diabetes, preterm delivery, intrauterine growth restriction, spontaneous abortion, and cesarean section [5–10]. However, other studies have demonstrated no association between vitamin D status and adverse pregnancy outcomes [11–12]. The majority of randomized control trials and observational studies measure vitamin D late in the pregnancy, when prenatal vitamins containing 400 IU of vitamin D have been prescribed [13]. Thus, we sought to determine if vitamin D deficiency in the first trimester of nulliparous patients is associated with clinical outcomes most associated with significant maternal and fetal morbidity and mortality: preeclampsia, gestational diabetes, preterm delivery, spontaneous abortion, and growth restriction. Nulliparous women were studied to decrease the effect of prior pregnancy complications on study outcomes. The objective of this study was to analyze the relationship between hypovitaminosis D in the first trimester and subsequent clinical outcomes in a population of nulliparous women.

Study Design

Participants

This study was approved by the Madigan Army Medical Center Institutional Review Board (Protocol #205031). Investigators adhered to the institutional policies for protection of human participants. All participants provided informed consent. Healthy, nulliparous women aged 18 years or older without a history of chronic medical conditions (e.g., preexisting diabetes mellitus (type 1 or 2), neurological disorders, cardiovascular anomalies, etc.) or infertility treatment were approached to participate in the study during their initial intake appointment at 8 to 12 weeks gestation by first day of the last menstrual period. All gestational ages were confirmed with either a first or a second trimester ultrasound, verified by chart review at study completion. Patients with predictors for hypovitaminosis D, such as anticonvulsant use, renal and cardiovascular disease, preexisting diabetes mellitus (type 1 or 2), were not eligible for recruitment at the beginning of the study. Women with a prior pregnancy that had progressed beyond the first trimester and resulted in a fetal loss or intrauterine fetal demise were also not approached for study enrollment. All patients were recruited from Madigan Army Medical Center, a tertiary military medical center with 2279 deliveries in 2014. All patients included in this study received their prenatal care and delivered at Madigan. Vitals including body mass index (BMI) and blood pressure were measured at initial and all subsequent obstetric appointments in accordance with

standard-of-care procedures. Additional demographic data was obtained including patient age, race, and tobacco use during pregnancy. Information extracted from delivery records included vital signs and gestational age at time of admission for delivery.

Based on clinical outcomes data, patients were segregated into cohorts of uncomplicated and complicated pregnancies. Complicated pregnancies were defined by the presence of adverse pregnancy outcomes including preeclampsia, gestational diabetes, preterm delivery, growth restriction, and spontaneous abortion. Preeclampsia and gestational diabetes were diagnosed as described in the American College of Obstetricians and Gynecologists practice bulletins [14–15]. Preterm delivery was considered as a spontaneous preterm birth less than 37 weeks gestational age due to preterm labor or preterm premature rupture of membranes and growth restriction was defined by estimated fetal weight less than the 10th percentile for gestational age. Spontaneous abortion was defined as spontaneous abortion in the first trimester after enrollment in the study [16–17].

Plasma preparation and vitamin D ELISA

Maternal blood was collected by venipuncture in vacutainer EDTA-containing tubes at 5 to 12 weeks gestational age and the date of the blood draw was recorded. Plasma was separated by centrifugation at 1500xg at 4°C in a Sorvall RC3C centrifuge (Sorvall Instruments, Thermo-Fisher Scientific, West Palm Beach, FL). Plasma was supplemented with protease inhibitors (Roche Diagnostics (1 836 145), Mannheim, Germany). Samples were aliquoted into multiple tubes and stored at -140°C. Total 25-OH vitamin D concentrations (ng/mL) were quantified in patient plasma samples by competitive enzymatic linked immunosorption assay (Catalog #DZ688A, Diazyme Laboratories, Poway, CA). Researchers were blinded to the pregnancy outcomes while performing the assays. Briefly, 25-OH vitamin D was dissociated from its serum transporter by acetonitrile extraction. Serum 25-OH vitamin D activated the enzyme beta-galactosidase and its substrate chlorophenol red-beta-D galactopyranoside, producing a colorimetric reaction product (chlorophenol red) detectable at 560nm by microplate reader (Synergy HT, Biotek, Winooski, VA). The amount of chlorophenol red was directly proportional to serum 25-OH vitamin D concentration. Concentrations were determined by interpolating to a standard curve generated by plotting absorbencies of calibrators at the following concentrations: 0, 14, 25, 45, 65, and 95 ng/ml (Diazyme #DZ688A-CAL). Assay quality control was assessed by measuring 25-OH vitamin D concentrations of assay controls run in parallel with patient samples. Assay fidelity was confirmed by liquid chromatography and tandem mass spectrometry as per the manufacturer's standard quality control procedures (Diazyme). Assay controls were 2 factory-supplied serum specimens containing approximately 24.4 ng/ml and 43.4 ng/ml 25-OH vitamin D (#DZ688A-CON, Diazyme; exact concentrations were lot-specific) as measured by an alternative ELISA strategy (Immunodiagnostic Systems, Scottsdale, AZ). Only assays in which controls were accurately measured from calibrator standard curves were included in the final data analysis. A quantitative comparative analysis of 29 standards (range of 20–113ng/ml) was undertaken by both liquid-chromatography/mass spectrometry (LC/MS) and the Diazyme immunoassay to determine the fidelity of the immunoassay relative to mass spectrometry.

The ELISA assay had an interassay variability of 9.3% and intraassay variability of 7.8%. Plasma vitamin D Diazyme vitamin D immunoassay performed comparably to LC/MS mass spectrometry. The comparative analysis resulted in a correlation coefficient of 0.95 between the two methods (-8.23% bias).

Categorization of vitamin D status

Recently published criteria from the Institute of Medicine were used to categorize vitamin D status by plasma 25-OH vitamin D concentrations: severe deficiency (<10 ng/ml [25nmol/L]); deficiency (< 20 ng/ml [50nmol/L]); insufficiency (21–29 ng/ml [51–74 nmol/L]); and sufficiency (\geq 30 ng/ml [75nmol/L] [18]).

Statistics

We estimated the approximate number of patients needed for adequate statistical power with an expected complication rate of 25% among all vitamin D categories. Assuming an α of 0.05, with statistical power of 80%, an effect size of 1.33 would require a sample size of at least 20 patients per category (sufficient, >30 ng/ml; insufficient, 21–29 ng/ml; or deficient, <20 ng/ml). However, we enrolled a total of 310 patients to account for possible attrition. Multivariable logistic regression was performed using Statistical Package for Social Sciences (SPSS) version 19. Chi-square analysis was performed on categorical variables and analysis of variance was performed on continuous variables. Chi square analysis was performed on the categorical variables (race, BMI at time of enrollment and plasma sampling, season, tobacco use, duty status, delivery mode) and analysis of variance (ANOVA) was performed on the continuous variables (maternal age and gestational age at intake). For logistic regression analysis, dependent variables were pregnancy clinical outcomes categorized as follows: no complications, preeclampsia, preterm delivery, intrauterine growth restriction, gestational diabetes, and spontaneous abortion. Independent variables were vitamin D category according to criteria recently published by the Institute of Medicine and Endocrine Society (i.e., sufficient, >30 ng/ml; insufficient, 21–29 ng/ml; or deficient, <21 ng/ml) [18–19]. Both unadjusted odds ratios and odds ratios adjusted for BMI, season, tobacco use, and ethnicity were calculated. Statistical significance was selected to be 0.05.

Results

Of the 315 patients approached for study participation, a total of 310 patients agreed to participate and gave informed consent according to the Institutional Review Board approval of the study protocol (response rate: 98.7%). A total of 235 patients were included in the final analysis (mean age 24.3 years, range 18–40 years). Of the original 310 patients recruited, 75 were not included in the study because they withdrew from the study ($n = 8$), did not deliver at our institution ($n = 44$), underwent elective pregnancy termination ($n = 3$), were lost to follow-up ($n = 18$) or were diagnosed with twin gestations after enrollment ($n = 2$) (attrition rate of 24.2%). Of the 235 patients included in the study, 41 of them had a prior pregnancy which had resulted in a spontaneous first trimester miscarriage or elective termination. Mean gestational age of recruitment and vitamin D plasma measurement was 62.2 ± 29.7 days (8.9 ± 4.2 weeks). A pregnancy was considered complicated if the patient was diagnosed with any of the following adverse pregnancy outcomes: preeclampsia, gestational diabetes, spontaneous preterm delivery, intrauterine growth restriction, or spontaneous abortion (Table 1). For the purposes of this study, patients who did not experience one of these complications were categorized as uncomplicated. Approximately 25% of patients had complicated pregnancies. Frequencies of pregnancy complications are tabulated in Table 1 and compared with predicted US and institutional frequencies for preeclampsia [20–26], preterm delivery [27–28], intrauterine growth restriction [29–30], gestational diabetes mellitus [31–36], and spontaneous abortion [37–38]. As shown in Table 1, three patients categorized in the spontaneous preterm delivery category had comorbidities including intrauterine growth restriction (IUGR) and gestational diabetes. These patients were not included in the IUGR or gestational diabetes categories for subsequent

Table 1. Frequency of clinical outcomes compared to national and global epidemiology.

| | Pregnancy Outcome n (% of 235 total) | Comorbidities | Predicted Population US (%) | Frequencies Global (%) | Madigan 2014 (%) |
|------------------------------|--------------------------------------|----------------------|-----------------------------|------------------------|------------------|
| None | 176 (75%) | – | – | – | – |
| Preeclampsia | 19 (8%) | 5 preterm deliveries | 3–10% [22,24–26] | 2–17% [20, 23] | 3% |
| Spontaneous preterm delivery | 10 (4%) | 2 IUGR, 1 GDM | 11.3% [27] | 9.6% [28] | 9% |
| IUGR | 9 (4%) | – | 8.6% [29] | 3–7% [30] | 0.6% |
| GDM | 5 (2%) | – | 4.6–9.2% [33] | 4.1–27.5% [36] | 4% |
| Spontaneous abortion | 16 (7%) | – | 8–20% [37,38] | | 6% |

IUGR, intrauterine growth restriction; GDM, gestational diabetes mellitus.

doi:10.1371/journal.pone.0123763.t001

statistical analysis. Similarly, the 5 patients with a medically indicated preterm delivery subsequent to preeclampsia were not included in the preterm delivery categories for subsequent analysis. The predicted adverse event frequencies were comparable to national and international epidemiological predictions (Table 1). The patient population comprised active duty military or dependent spouses (Table 2). Information on patient time in the area prior to pregnancy, dietary intake of vitamin D, and sun exposure was not available. All patients were prescribed prenatal vitamins (approximately 400 IU/day) at their initial intake appointment, but compliance rates on use were unknown. When actual plasma vitamin D concentrations were determined for the 235 patients included in the final analysis, technical reproducibility among replicates was assessed by calculating the coefficient of variation (CV) among calibrators, controls, and samples. CVs were 15.8% for calibrators, 8.5% for assay controls, and 7.8% for patient plasma samples. Ten percent of our study population was vitamin D deficient with a serum concentration less than 20 ng/mL (50 nmol/L); 60% were vitamin D insufficient (serum concentrations between 20–29 ng/mL or 50–74 nmol/L); and 30% of our study population were vitamin D sufficient with serum concentrations above 30 ng/mL (75 nmol/L) (Table 2). None of our patients were classifiable as severely vitamin D deficient (<10 ng/ml or 25 nmol/L). The mean serum 25-OH vitamin D concentration for our study population was 27.6 ng/mL (range 13–71.6 ng/mL).

Forty-seven percent of our study population was of normal weight with a BMI <25 (Table 2). Thirty-five percent were overweight with a BMI at intake between 25–30, and about 18% of our patients were obese with a BMI >30. Most of our study population was Caucasian (78%), with 7% African American, 3% Asian, and 12% other, unspecified races. Thirty-eight percent of the blood draws occurred in the winter, 19% in the spring, 22% in the summer, and 21% in the fall. About 6% of our study population used tobacco during pregnancy. Statistical associations between vitamin D categorization and demographic variables are depicted in Table 2. Race was the only statistically different variable among the vitamin D groups, with significantly more African American, Asian, and other unspecified racial groups in the vitamin D deficient category compared to the insufficient and sufficient groups (p = 0.03).

The distribution of clinical outcomes among the vitamin D categories is depicted in Table 3. Study population percentages and with medians and ranges of vitamin D measured in plasma were calculated. Preeclampsia was sub-stratified into severe, early onset preeclampsia and mild, late-onset preeclampsia.

Plasma vitamin D concentrations were subdivided into 3 categories based on the Institution of Medicine and Endocrine Society criteria for vitamin D categorization: sufficient (>30ng/ml [75nmol/L]), insufficient (21-29ng/ml [50-75nmol/L]), or deficient [<50nmol/L]. None of our

Table 2. Demographics of the study population by vitamin D classification.

| | Total n = 235 (%) | Vitamin D Sufficient (>30 ng/ml) n = 70 (30%) | Vitamin D Insufficient (21–29 ng/ml) n = 141 (60%) | Vitamin D Deficient (<20 ng/ml) n = 24 (10%) | p value** |
|--|----------------------|---|--|--|------------------|
| Age (years)* | 24.3 ± 4.4 | 24.5 ± 4.2 | 24.6 ± 4.5 | 22.5 ± 3.7 | 0.08 |
| Gestational Age at Analysis (days)* | 62.2 ± 29.7 | 61.8 ± 13.7 | 62.2 ± 36.9 | 63.0 ± 11.2 | 0.99 |
| | n (% of 235) | n (% of 70) | n (% of 141) | n (% of 24) | p value** |
| Race | | | | | 0.03 |
| Caucasian | 183 (77.9) | 57 (81.4) | 114 (80.9) | 12 (50) | |
| African American | 16 (6.8) | 3 (4.3) | 10 (7.1) | 3 (12.5) | |
| Asian | 6 (2.6) | 1 (1.4) | 3 (2.1) | 2 (8.3) | |
| Other | 30 (12.8) | 9 (12.9) | 14 (9.9) | 7 (29.2) | |
| Body Mass Index | | | | | 0.36 |
| <25 | 111 (47.2) | 39 (55.7) | 62 (44) | 10 (41.7) | |
| 25–30 | 82 (34.9) | 21 (30.0) | 50 (35.5) | 11 (45.8) | |
| >30 | 42 (17.9) | 10 (14.3) | 29 (20.5) | 3 (12.5) | |
| Season | | | | | 0.22 |
| Winter | 89 (37.9) | 22 (31.4) | 61 (43.3) | 6 (25.0) | |
| Spring | 45 (19.1) | 16 (22.9) | 25 (17.7) | 4 (16.6) | |
| Summer | 53 (22.6) | 20 (28.6) | 26 (18.4) | 7 (29.2) | |
| Fall | 48 (20.4) | 12 (17.1) | 29 (20.6) | 7 (29.2) | |
| Mode of Delivery | | | | | 0.41 |
| Vaginal | 155 (66.0) | 47 (67.1) | 95 (67.4) | 13 (54.2) | |
| Cesarean section | 63 (26.8) | 18 (25.7) | 36 (25.5) | 9 (37.5) | |
| IUFD or SAB | 17 (7.2) | 5 (7.1) | 10 (7.1) | 2 (8.3) | |
| Duty Status | | | | | 0.12 |
| Active Duty | 83 (35.3) | 22 (31.4) | 48 (34.0) | 13 (54.2) | |
| Dependent | 152 (64.6) | 48 (68.6) | 93 (66.0) | 11 (45.8) | |
| Tobacco Use | | | | | 0.67 |
| Yes | 15 (6.4) | 3 (4.3) | 10 (7.1) | 2 (8.3) | |
| No | 220 (93.6) | 67 (95.7) | 131 (92.9) | 22 (91.7) | |

*Expressed in mean ± standard deviation

** , p value calculated by analysis of variance for continuous variables and chi-square analysis for categorical variables

IUFD, intrauterine fetal demise; SAB, spontaneous abortion.

doi:10.1371/journal.pone.0123763.t002

patients were categorized as severely deficient (<10ng/ml [25nmol/L]) (Table 4). Because there were too few patient in the category of vitamin D deficient (<20ng/ml [50nmol/L]) for meaningful statistical analysis, patients categorized as either vitamin D insufficient or deficient were combined into one category (insufficient/deficient, <30ng/ml [75nmol/L]) for subsequent statistical analysis (Table 5).

There was no association between vitamin D deficiency in the first trimester and adverse pregnancy outcomes with an adjusted odds ratio of 1.01 (p value 0.738, 95% CI 0.947–1.164). Logistic regression was performed on combined outcomes and individual outcomes separately. There was no association between first trimester vitamin D levels and subsequent development of combined complications, or complications sub-stratified into preeclampsia, gestational diabetes, preterm delivery, intrauterine growth restriction (IUGR), or spontaneous abortion when each outcome was evaluated individually (Table 5). Odds ratios were adjusted for BMI,

Table 3. Plasma vitamin D concentrations in women according to clinical outcome.

| | n (% of 235 total) | median vitamin D concentration in ng/ml (range) |
|------------------------------|--------------------|---|
| No complications | 176 (75) | 27.4 (13.0–71.6) |
| All complications | 59 (25) | 26.5 (13.2–42.3) |
| Preeclampsia | 19 (8) | 26.5 (16.1–35.9) |
| Severe, early onset | 5 (2) | 27.1 (18.0–33.8) |
| Mild, late onset | 14 (6) | 27.7 (16.1–35.9) |
| Spontaneous Preterm delivery | 10 (4) | 28.4 (19.9–42.3) |
| IUGR | 9 (4) | 27.0 (16.6–38.8) |
| GDM | 5 (2) | 22.2 (20.5–31.9) |
| Spontaneous Abortion | 16 (7) | 25.1 (13.2–38.8) |

IUGR, intrauterine growth restriction; GDM, gestational diabetes mellitus.

doi:10.1371/journal.pone.0123763.t003

ethnicity, tobacco use, and season. There was no association between first trimester vitamin D levels and subsequent development of composite adverse pregnancy complications (including preeclampsia [severe, early onset or mild, late onset], gestational diabetes, preterm delivery, growth restriction, or spontaneous abortion (combined outcomes; $p > 0.05$ in [Table 5](#)).

Discussion

This study investigated the prevalence of hypovitaminosis D in young (24.3 ± 4.4 years), nulliparous women at a tertiary military medical facility in the Pacific Northwestern United States at an average gestational age of 8.9 ± 4.2 weeks. Our study is distinctive as we examined a healthy population of women who should be at relatively lower risk for pregnancy complications. These women did not have preexisting hypertension, diabetes, or other medical problems that would increase their risk for pregnancy complications or vitamin D deficiency. Although we recruited only young, nulliparous, previously healthy women, the majority of our patients were either vitamin D deficient (10%, <20 ng/ml) or vitamin D insufficient (60%, $20\text{--}29$ ng/ml) according to the criteria published by the Institute for Medicine.¹⁸ Of the 235 patients included in the study, 41 of them had a prior pregnancy which had resulted in a spontaneous first

Table 4. Distribution of clinical outcomes in women categorized as Vitamin D deficient, insufficient, or sufficient.

| Vitamin D Category | No Complications n (% of 176) | All Complications n (% of 59) | Preeclampsia n (% of 19) | Spontaneous Preterm delivery n (% of 10) | IUGR n (% of 9) | GDM n (% of 5) | Spontaneous Fetal loss n (% of 16) |
|---|-------------------------------|-------------------------------|--------------------------|--|-----------------|----------------|------------------------------------|
| Deficient (<20 ng/ml) (n = 24) | 18 (10) | 6 (10) | 2 (11) | 1 (10) | 1 (11) | 0 (0) | 2 (13) |
| Insufficient (21–29 ng/ml) (n = 141) | 105 (60) | 36 (61) | 10 (53) | 6 (60) | 7 (78) | 3 (60) | 10 (63) |
| Sufficient (>30 ng/ml) (n = 70) | 53 (30) | 17 (29) | 7 (37) | 3 (30) | 1 (11) | 2 (40) | 4 (25) |

IUGR, intrauterine growth restriction; GDM, gestational diabetes mellitus.

doi:10.1371/journal.pone.0123763.t004

Table 5. Logistic regression analysis of clinical outcomes in women categorized as vitamin D sufficient compared to vitamin D insufficient or deficient women.

| | Vitamin D Sufficient (≥ 30 ng/ml) n (% of 70) | Vitamin D Insufficient or Deficient (< 30 ng/ml) n (% of 165) | Adjusted** OR (95% CI)* |
|------------------------------|--|---|-------------------------|
| No complications | 53 (75.7) | 123 (74.5) | - |
| All complications | 17 (24.2) | 42 (25.5) | 1.01 (0.947–1.164) |
| Preeclampsia | 7 (10) | 12 (7.2) | 1.36 (0.48–3.88) |
| Spontaneous Preterm delivery | 3 (4.3) | 7 (4.2) | 0.78 (0.17–3.55) |
| IUGR | 1 (1.4) | 8 (4.8) | 0.33 (0.38–2.83) |
| GDM | 2 (2.9) | 3 (1.8) | 2.60 (0.28–27.24) |
| Spontaneous Abortion | 4 (5.7) | 12 (7.2) | 0.65 (0.18–2.28) |

* , note: none of the differences were $p < 0.05$ by logistic regression (chi-square analysis)

** , Adjusted for BMI, season, ethnicity, and tobacco use

OR, odds ratio

IUGR, intrauterine growth restriction; GDM, gestational diabetes mellitus.

doi:10.1371/journal.pone.0123763.t005

trimester miscarriage or elective termination. As these prior losses were not associated with a diagnosis of infertility, recurrent pregnancy loss or other medical conditions, we felt there would be minimal confounding by including them in our study. We found no association between hypovitaminosis D (plasma levels of < 30 ng/ml) and the combined clinical outcomes of preeclampsia, preterm delivery, intrauterine growth restriction, gestational diabetes, or spontaneous abortion in this patient population. This study was not powered to detect associations between vitamin D status and these individual pregnancy complications. Fig 1 compares our study population with recently published cohort studies and randomized controlled trials (reviewed in Christesen [2012] and Wei [2013]) [13, 39].

Risk factors for vitamin D deficiency include inadequate sun or UV light exposure, physiologic factors (such as dark skin pigmentation, obesity, pregnancy, certain medical problems), and low vitamin D intake [2–3]. The prevalence of vitamin D deficiency in reproductive aged women appears to be increasing worldwide over the past two decades.^{1,3,13} Recent studies indicate the prevalence of vitamin D insufficiency and deficiency in nulliparous women, a result corroborated by our study [40]. The etiology of the decline of vitamin D status in women is likely multifactorial, but is due in part to increasing BMI and increased sun protection, coupled with decreased dietary intake of vitamin D, such as milk and other dairy products. Unfortunately, the increasing use of multivitamins and mineral dietary supplements in younger to older adults does not appear to be associated with a corresponding increase in serum 25-OH vitamin D concentrations [3, 19, 41]. Recent clinical guidelines suggest that doses of vitamin D greater than 600 IU/day may be needed to maintain adequate plasma levels greater than 30 ng/ml (75 nmol/L), but the appropriate standard is still debated [19, 42–43]. In recent randomized controlled studies, loading doses as high as 1000–4000 IU/day were needed before adequate vitamin D plasma concentrations were measured [18–19, 44].

Other risk factors for vitamin D deficiency include ethnicity, as darker skinned individuals have decreased vitamin D₃ synthesis in response to ultraviolet light. Vitamin D deficiency has been proposed as a possible biologic explanation for racial disparities noted in adverse pregnancy outcomes including preeclampsia, spontaneous preterm birth, gestational diabetes and growth restriction [5]. Because vitamin D deficiency is so widespread, determining whether vitamin D deficiency is associated with adverse pregnancy outcomes is an area of major public health concern. It is unclear whether vitamin D deficiency is causal in the disparities in adverse

| Clinical outcome | Study | Maternal age* | Parity** (% Nulliparous) | Geographical Location | Gestational age of Vitamin D analysis | Number of subjects | Vitamin D Threshold | | |
|-------------------------------------|-------------------------------|---------------|-----------------------------|---|--|------------------------------|------------------------|----------------|--------------|
| | | | | | | | Sufficient | Insufficient | Deficient |
| Preeclampsia | present study | 24.3 ± 4.4 | 100% | Pacific Northwestern United States | 4-12 weeks | 235 | ≥75 nmol/L | 50-74 nmol/L | ≤50 nmol/L |
| | Hausman 2009 | -- | 100% | Norway | -- | 23,423 | -- | -- | -- |
| | Bodnar 2007 | -- | 100% | Northeastern United States | <12 weeks | 55 cases, 219 controls | -- | -- | -- |
| | Baker 2011 | 33-35 | 47% | United States, North Carolina | 11-14 weeks | 51 cases, 204 controls | ≥75 nmol/L | 50-74 nmol/L | ≤50 nmol/L |
| | Robinson 2010 | -- | 30% | United States, South Carolina | -- | 50 cases, 100 controls | -- | -- | -- |
| | Woodham 2011 | 29 | 20% | United States, North Carolina | 15-20 weeks | 41 cases, 123 controls | -- | -- | -- |
| | Pove 2010 | 28.9 - 30.4 | 58% | United States, Massachusetts | <12 weeks | 39 cases, 131 controls | >20 nmol/L | 10-15 nmol/L | <10 nmol/L |
| | Shand 2010 | 94% >25 | 43% | Canada, British Columbia | 10-20 weeks | 28 cases, 193 controls | ≥75 nmol/L | 50-74 nmol/L | ≤50 nmol/L |
| | Azar 2011 | 31.7 | 55% | United States | <13 weeks | 23 cases, 24 controls | ≥30 nmol/L | 20-30 nmol/L | <20 nmol/L |
| | Hakala 2000 | 22.7 - 24.5 | N+M | Mexico | 39.6 - 40 weeks | 36 cases, 36 controls | -- | -- | -- |
| | Hypponen 2007 | -- | 31% | Finland | -- | 2950 | -- | -- | -- |
| | Fernandez-Alonso 2012 | -- | N+M | Almeria, Spain | 11-14 and 36-39 weeks | 466 | ≥30 nmol/L | 20-30 nmol/L | <20 nmol/L |
| | Wei 2012 | 30-31 | 80% | Canada | 12-13 and 24-26 weeks | 32 cases, 665 controls | ≥75 nmol/L | 50-74 nmol/L | ≤50 nmol/L |
| | Magtibog 2008 | 25-30 | N+M | Tehran, Iran | 24-28 weeks | 52 cases, 689 controls | >37.9 nmol/L | 25-34.9 nmol/L | <24.9 nmol/L |
| | Gestational diabetes mellitus | Zhang 2008 | 33.1 - 34.1 | 50% | Washington state, USA | 16 weeks | 57 cases, 114 controls | >30 nmol/L | 20-29 nmol/L |
| Parke 2012 | | 34.3 | N+M | Toronto, Canada | 15-17 weeks | 116 cases, 219 controls | -- | -- | -- |
| Clifton-Bligh 2008 | | 32.6 ± 5.1 | N+M | Sydney, Australia | >90% were 2-3 trimester | 81 cases, 184 controls | ≥75 nmol/L | 50-74 nmol/L | ≤50 nmol/L |
| Maigbo 2011 | | 33.1 - 34.2 | 42% | London, UK | <12 weeks | 90 cases, 158 controls | ≥75 nmol/L | 50-74 nmol/L | ≤50 nmol/L |
| Farrar 2009 | | 23.7 | N+M | Mysore, S India | 30 weeks | 39 cases, 520 controls | >50 nmol/L | -- | ≤50 nmol/L |
| Shand 2010 | | 94% >25 | 43% | Vancouver, Canada | 19 weeks | 28 cases, 193 controls | ≥75 nmol/L | 50-74 nmol/L | ≤50 nmol/L |
| Baker 2011 | | 33-35 | 47% | North Carolina, USA | 11-14 weeks | 60 cases, 120 controls | ≥75 nmol/L | 50-74 nmol/L | ≤50 nmol/L |
| Mitra 2009 | | 24.6 | N+M | Dar es Salaam, Tanzania | 12-27 weeks | 894 (all HIV-infected) | >32 nmol/L | <32 nmol/L | -- |
| Shiba 2011 | | 31.4 | N+M | Tokyo, Japan | after 30 weeks | 14 cases, 79 controls | -- | -- | -- |
| Burris 2012 | | 32 | 47% | Massachusetts, USA | 2-6 weeks | 2128 | ≥75 nmol/L | 50-74 nmol/L | ≤50 nmol/L |
| Baker 2012 | | 33-35 | 43% | Boston, Massachusetts, USA | 24-28 weeks | 4225 | ≥75 nmol/L | 50-74 nmol/L | ≤50 nmol/L |
| Fernandez-Alonso 2012 | | -- | N+M | Almeria, Spain | 11-14 and 36-39 weeks | 466 | ≥30 nmol/L | 20-30 nmol/L | <20 nmol/L |
| Kohasari 2008 | | 29 | N+M | Turkey | 10-19 | 72 | -- | -- | -- |
| McLeod 2011 | | 29 | N+M | Brisbane, Australia | 34-37 | 399 | -- | -- | -- |
| Sawidou 2011 | | 31-35 | 48% | London, UK | 11-13 | 1200 | ≥30 nmol/L | <30 nmol/L | -- |
| Soheib/Alah 2010 | 27 | N+M | Iran | 24-28 | 204 | >30 nmol/L | 20-29 nmol/L | <20 nmol/L | |
| Moller 2012 | 29 | 69% | Aarhus, Denmark | pre conception, trimester 1, 2, 3, postpartum | 50 | >80 nmol/L | 50-80 nmol/L | <50 nmol/L | |
| Infertility or Spontaneous Abortion | Oken 2007 | 29% 20 - <30 | 49% | Massachusetts, USA | 10 and 26-28 weeks | 1718 | -- | -- | -- |
| | Johnson 2011 | 24.8 - 30.1 | 28% | South Carolina, USA | <14 weeks | 494 | >32 nmol/L | 20-32 nmol/L | <20 nmol/L |
| | Tavakoli 2011 | <40 | N+M | Tehran, Iran | preconception | 8 cases, 8 controls | -- | -- | -- |
| | Christian 2008 | 38% > 25 | 26% | Nepal | -- | 4926 | -- | -- | -- |
| | Duroso 2012 | 26 | N+M | Nashville, Tennessee, USA | delivery | 160 | -- | -- | <20 nmol/L |
| | Perez-Ferre 2012 | 33 | N+M | Madrid, Spain | 24-28 | 266 | -- | -- | <20 nmol/L |
| Small for gestational age IUGR | Okan 2010 | 34 | -- | New York, USA | pre-pregnancy | 84 women (IVF) | >30 nmol/L | 20-30 nmol/L | <20 nmol/L |
| | Brough 2010 | 27.9 - 28 | 52% | East London, UK | 20, 26, 34 weeks | okcebo 195, intervention 207 | >50 nmol/L | 25-50 nmol/L | <25 nmol/L |
| | Robinson 2011 | 27.9 - 28.4 | 25-36% | South Carolina, USA | <34 weeks | 56 | -- | -- | -- |
| | Bodnar 2010 | 52% 20-29 | 100% | Pittsburgh, Pennsylvania, USA | <22 weeks | 412 | >75 nmol/L | 37.5-75 nmol/L | <37.5 nmol/L |
| | Leffelaar 2010 | 89% >24 | 57.1 | Amsterdam, The Netherlands | 12 weeks | 3730 | ≥50 nmol/L | 30-49.9 nmol/L | ≤29.9 nmol/L |
| | Burris 2012 | 30-33 | 48% | Boston, Massachusetts, USA | 26-28 weeks | 10 | ≥75 nmol/L | 50-74 nmol/L | ≤50 nmol/L |
| RCT or intervention study | Germond 2013 | 62% 20-29 | 33% | Pittsburgh, Pennsylvania, USA | <26 weeks | 2146 | >37.5 nmol/L | <37.5 nmol/L | -- |
| | Brooke 1980 | 24 | 53-58% | United Kingdom | 28-32 weeks | 126 | -- | -- | -- |
| | Mallet 1986 | 25-26 | N+M | Northwest France | delivery | 77 | -- | -- | -- |
| | Delvin 1986 | -- | N+M | Lyon France | cord blood and 4 days postpartum | 44 | -- | -- | -- |
| | Marva 1988 | 24.0-24.1 | N+M | Northern India | -- | 200 | -- | -- | -- |
| | Sahu 2009 | -- | N+M | Northern India | delivery | 84 | -- | -- | -- |
| Yu 2009 | -- | N+M | United Kingdom | 27 weeks and delivery | 180 | >50 nmol/L | 25-50 nmol/L | <25 nmol/L | |
| Hollis 2011 | 26-27 | N+M | South Carolina, USA | 12-16 weeks, then monthly until delivery | 350 | >80 nmol/L | 50-80 nmol/L | <50 nmol/L | |

* median or mean; ranges listed when ages were separated by cohort
 **N: nulliparous; M: multiparous; percent unspecified

Fig 1. Comparison of study parameters in recent vitamin D observational and intervention studies.

doi:10.1371/journal.pone.0123763.g001

pregnancy outcomes between Caucasians and African Americans. Out of 235 patients in our study, only about 7% were African American and thus we were not powered enough to examine this significance. However, even after adjusting for ethnicity in our analysis, there was still no significant difference between hypovitaminosis D and clinical outcome.

There are two forms of vitamin D in the body: vitamin D2 and vitamin D3. Measures of blood levels reflect both dietary intake as well as synthesis from exposure to the sun. The liver converts both forms of vitamin D to 25-OH vitamin D, which is the specific metabolite measured to determine vitamin D status. These levels are considered to be an accurate representation of the vitamin D status of an individual [1, 45]. A serum level of at least 20 ng/mL (50 nmol/L) is needed to avoid bone problems, and the optimal level for promoting health was recently published as 30 ng/mL (75 nmol/L) [2, 5, 18-19]. However, it is important to note that these thresholds were derived from a population of nonpregnant individuals and an optimal serum level during pregnancy has not been established.

Season and latitude dramatically alter vitamin D3 production [46-47]. In wintertime, the angle of the sun is so oblique that latitudes greater than 35 degrees receive almost no ultraviolet

rays capable of stimulating vitamin D₃ synthesis. As our study center is around 47 degrees latitude and 38% of our first trimester blood draws occurred in the winter, this certainly could contribute to the prevalence of vitamin D deficiency in our population. In addition, the Pacific Northwest experiences fewer sunny days than the rest of the country, with only 46% of daylight hours that are predominantly sunny or partly cloudy. For example, the greater Seattle area experiences an average of 8 and a half hours of daylight hours during December but only roughly 2 hours during the day are considered “sunny”. Compare this to South Carolina, which experiences over 6 hours of sunshine out of almost 10 hours of daylight during the same month. Unfortunately, data on individual daily sun exposure, travel and dietary intake are not available [48–49]. Other limitations and sources of possible bias for this study include low generalizability (small cohort of patients in the Pacific Northwestern United States), small sample size, self-selection of study subjects, and the high loss-to-follow-up rate in our study population. Information on the job description of active duty women was unavailable for this study, as was data on physical activity and location of duty station influencing sun exposure (i.e., indoors vs. outdoors). Body mass index in this population may not necessarily be indicative of overweight or obese given that some active duty women may have more muscle mass contributing to BMI measurement.

Several recent randomized controlled trials and observational cohort studies investigate a possible association between hypovitaminosis D and adverse clinical outcome in pregnancy (reviewed in Christesen [2012]) [13]. The role vitamin D plays in adverse clinical outcomes such as preeclampsia, gestational diabetes, preterm labor, growth restriction, and spontaneous abortion is speculative, but may involve vitamin D’s regulation of placental trophoblast invasion and angiogenesis, in addition to its anti-inflammatory properties [5]. For example, there is some evidence vitamin D affects transcription and function of genes responsible for trophoblast invasion and angiogenesis, two factors critical for placenta development. Vitamin D deficiency may predispose individuals to abnormal trophoblast invasion, reduced placental perfusion, and the subsequent cascade of events resulting in preeclampsia. Maternal vitamin D deficiency may also predispose women to an increased inflammatory response that characterizes preeclampsia, especially severe, early onset disease (Reviewed in Christesen [2012] and Wei [2013]) [13, 39]. In our cohort of young, nulliparous patients, we had a preeclampsia rate of 8%. However, vitamin D deficiency did not increase the odds of preeclampsia, even when stratifying by disease severity. This discrepancy may be explained in part by differences in our study population relative to other published cohort studies, including difference in parity, maternal age at intake, and/or gestational age of specimen collection (Fig 1) [6, 10–11, 40, 50–70]. During pregnancy, 25-OH vitamin D diffuses across the placenta and the fetus relies entirely on the vitamin D stores of the mother: if the mother is deficient, so is the fetus [5]. Thus, in combination with its immunomodulatory and anti-inflammatory properties, vitamin D may play a potential role in the prevention of preterm birth and small-for-gestational-age neonates. Vitamin D deficiency also has been shown to increase insulin resistance and reduce insulin secretion, which has shown to be a risk factor for gestational diabetes [5, 71–72]. However, whether vitamin D deficiency is a risk factor for gestational diabetes in itself or if vitamin D supplementation can prevent GDM is yet unknown [72].

Although vitamin D has a biologically possible role in all the aforementioned disorders, this was not demonstrated in our population as vitamin D deficiency did not increase the odds for adverse pregnancy outcome in our study. Study-specific parameters such as maternal demographics and parity may account for some of these differences in our cohort relative to published studies (Table 2 and Fig 1). For example, we had a lower incidence of gestational diabetes in our study population compared to what is reported in the literature [31–35, 73–75]. Risk factors for gestational diabetes include women older than 25, obesity (BMI >30), history

of a large baby or previously affected pregnancy, and women who are African American, American Indian, Asian American, Hispanic, Latina, or Pacific Islander [14]. We feel our low incidence of GDM is likely due to the young age (average age 24 years old), nulliparity, relatively low rate of obesity (17.9%), and predominantly white ethnicity (77%) of our study population. Obesity is also a known risk factor for vitamin D deficiency [73]. We reviewed the BMI of our study participants to see if there was any association between vitamin D deficiency classification and BMI. Out of the 235 patients analyzed, 42 (17.9%) had a prepregnancy BMI >30 and were considered obese. However, there was no significant difference in the distribution of these patients among the various vitamin D categories, with 10/70 (14.3%) women who were vitamin D sufficient, 29/141 (20.5%) vitamin D insufficient, and 3/24 (12.5%) vitamin D deficient ($p = 0.22$) (Table 2).

Our nulliparous patient population is also at different risk for certain pregnancy complications, such as preeclampsia and preterm delivery, compared to their multiparous counterparts. The prevalence of preeclampsia in the United States is about 3.4 percent, but 1.5-fold to 2-fold higher in first pregnancies [22]. In fact, most cases of preeclampsia occur in nulliparous women without significant medical history or risks factors [15]. Our nulliparous population may also be at lower risk for preterm delivery as one of the strongest clinical risk factors for preterm birth is a prior preterm birth [76]. Behavioral risk factors for preterm birth include low maternal prepregnancy weight, smoking, substance abuse, and short interpregnancy interval. Only three of our patients had a BMI less than 19; all three of these women had full term deliveries. Additionally, our maternal tobacco use rate was only 6.4%, compared to national averages closer to 13% [77]. All of these factors may have contributed to our low rate of preterm delivery compared to nationally reported averages.

Conclusion

Vitamin D deficiency was not associated with the composite clinical outcomes of preeclampsia, preterm delivery, intrauterine growth restriction, gestational diabetes, or spontaneous abortion in this study population of nulliparous military members and their beneficiaries. This study was not powered to detect associations between vitamin D status and these individual complications of pregnancy. However, the high percentage of affected individuals highlights the prevalence of vitamin D deficiency and insufficiency in young, healthy reproductive-aged women in our study population. Vitamin D deficiency is a growing topic of interest around the world. Although our study did not demonstrate an association of vitamin D plasma concentration with select clinical outcomes, the prevalence of sub-optimal vitamin D concentrations in patient plasma emphasizes the need for continued research on hypovitaminosis D in pregnancy and reproductive health.

Acknowledgments

We would like to thank Diazyme Laboratories for their technical assistance with the 25-OH Vitamin D enzyme immunoassay as well as for their review of our manuscript.

Disclaimer: The views expressed in this presentation are those of the authors and do not reflect the official policy of the Department of the Army, the Department of Defense or the U.S. Government.

Author Contributions

Conceived and designed the experiments: SFN DT PN DI. Performed the experiments: SFN DT DI. Analyzed the data: SFN RH DI. Contributed reagents/materials/analysis tools: SFN DT DI. Wrote the paper: SFN. Reviewed and revised the manuscript: PN.

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