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DOCTORAL THESIS

**The Implications of Vitamin D in Pregnancies Associated
with Preeclampsia**

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STUDY 1: THE INFLUENCE OF MATERNAL VITAMIN D SUPPLEMENTATION IN PREGNANCIES ASSOCIATED WITH PREECLAMPSIA: A CASE-CONTROL STUDY.

CONTEXT

Preeclampsia is a pregnancy-related condition characterized by high blood pressure and proteinuria, posing significant risks to maternal and perinatal health. It affects approximately 2-10% of pregnancies worldwide. Proteinuria, marked by elevated protein levels in urine, remains a key diagnostic criterion. Vitamin D deficiency is a common concern during pregnancy, affecting up to 30% of African-American and 5% of Caucasian pregnant women in certain regions. This deficiency has been linked to preeclampsia development, with global prevalence ranging from 18-84% due to various factors.

Vitamin D₃, produced when the skin is exposed to UV-B radiation, plays a crucial role in bone health and calcium metabolism. It is converted to its active form, 1,25-dihydroxy vitamin D₃, which regulates gene expression and impacts multiple organs, including the placenta. Risk factors for preeclampsia include diabetes, chronic hypertension, renal conditions, obesity, and a personal or family history of the condition. Vitamin D deficiency can lead to adverse pregnancy outcomes, including low birth weight and maternal complications, though clinical studies have yielded conflicting results. Low vitamin D levels may disrupt the balance between immune responses, potentially contributing to preeclampsia. Given the suspected link between vitamin D deficiency and preeclampsia, this study aimed to retrospectively assess the association between preeclampsia risk in pregnant women and their vitamin D supplementation. It hypothesized that higher vitamin D supplementation doses and serum 25(OH)D levels would be associated with a reduced risk of preeclampsia.

The primary objectives of this study are to retrospectively investigate the relationship between vitamin D supplementation and serum 25(OH)D levels in pregnant women at risk of preeclampsia and to assess whether higher doses of vitamin D supplementation and elevated serum 25(OH)D levels are associated with a reduced risk of developing preeclampsia. Additionally, this study aims to explore the potential impact of vitamin D supplementation on various adverse pregnancy outcomes, including gestational diabetes, low birth weight, premature labor, and the need for cesarean section, and to examine whether vitamin D deficiency alters the immune response balance, particularly the expression of Th1 cytokines, which may be linked to the development of preeclampsia.

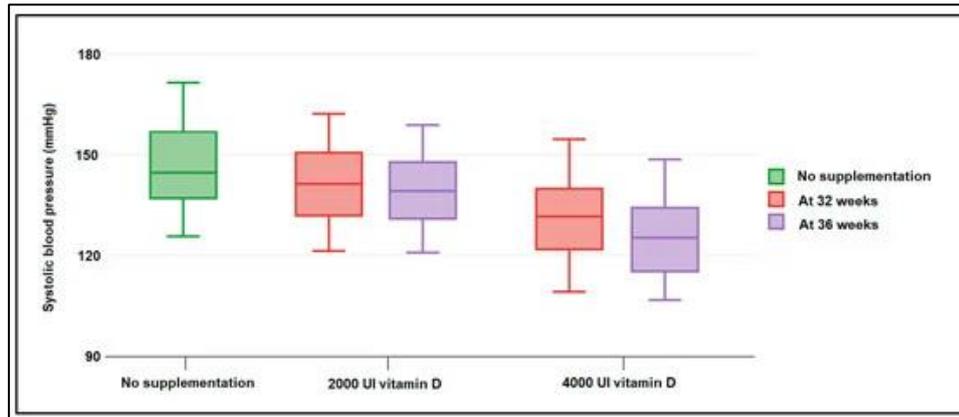
RESULTS

The study analyzed three groups based on vitamin D supplementation during pregnancy. The groups consisted of 59 pregnant women with a history of preeclampsia who did not take vitamin D during their second pregnancy, 63 who took a daily dose of 2000 units of vitamin D during the first trimester, and 76 who took a daily dose of 4000 UI. When examining maternal backgrounds, there were no significant differences in age, number of previous children, urban or rural dwelling, employment status, education, income, and civil status among the groups. Most participants fell within the 25 to 34 age range and had a child from a previous pregnancy.

A closer look at the pregnancy characteristics showed that anemia was the most common issue across all groups, with a 35% average prevalence. The rate of gestational diabetes mellitus, a risk factor for preeclampsia, was 7.5%. Obesity was the most prevalent comorbidity, with varying frequencies across groups, followed by cardiovascular disease. When assessing neonatal outcomes, there were significant differences in premature births and antibiotic treatment needs for the newborn based on vitamin D supplementation. Those not supplementing with vitamin D had higher prematurity and antibiotic treatment rates compared to the supplemented groups.

Further analysis at 32 and 36 weeks of pregnancy indicated a stronger association of vitamin D deficiency in those who didn't supplement. Specifically, 20.3% in the non-supplementation group showed moderate to severe vitamin D deficiency, compared to 12.7% in the low dose and 6.6% in the high dose groups. Hypertension was more prevalent in the no-supplementation group at 32 weeks. Risk analysis further solidified the connection between vitamin D levels and the likelihood of developing preeclampsia in the second pregnancy. Insufficient vitamin D levels increased the likelihood of preeclampsia, as did having a history of multiple pregnancies, gestational diabetes, and cardiovascular comorbidities.

Figure 1 – Maternal proteinuria (g/L) measured at 32 and 36 weeks of gestation.



CONCLUSIONS

The study underscores the crucial role vitamin D plays in maintaining placental function during the later stages of pregnancy. Specifically, women who are past the 32-week mark of their gestation period and exhibit signs of vitamin D deficiency are at a heightened risk for placenta-mediated hypertension or preeclampsia, a potentially life-threatening condition. This connection between vitamin D status and placental function is significant. A deficiency in vitamin D can compromise the placenta's ability to function optimally, leading to complications such as preeclampsia. This revelation not only sheds light on the importance of adequate vitamin D levels during pregnancy but also positions vitamin D as a potential preventive measure against such complications.

Given the gravity of the consequences associated with preeclampsia and the implications of the study's findings, there's a clear need for further research. Vitamin D supplementation could emerge as a viable and straightforward intervention for preventing preeclampsia, provided its efficacy and safety are firmly established. Therefore, rigorous, randomized controlled trials are of paramount importance at this juncture. These trials will help confirm whether vitamin D supplementation can indeed serve as a preventive measure against preeclampsia and ensure that it's a safe and effective intervention for expectant mothers..

STUDY 2: A PROSPECTIVE ANALYSIS OF VITAMIN D LEVELS IN PREGNANT WOMEN DIAGNOSED WITH GESTATIONAL HYPERTENSION AFTER SARS-COV-2 INFECTION.

CONTEXT

It is typical for pregnant women to have low amounts of 25-hydroxyvitamin D circulating in their blood. The active form of vitamin D has the ability to suppress renin biosynthesis and vascular smooth muscle cell proliferation and to regulate the transcription of genes that are linked to placental invasion, normal implantation, and angiogenesis. Throughout pregnancy, the vitamin D physiology of the mother is changed, resulting in elevated levels of the vitamin D binding protein. Current theories suggest that the rise in 1,25(OH)₂D is a biological response created to allow immunological tolerance via vitamin D pathways at the maternal–fetal interface, hence promoting healthy placentation.

Hypertensive disorders during pregnancy are a leading contributor to severe acute morbidity, long-term impairment, and death in both the mother and the developing baby. It is believed that ten percent of pregnant women throughout the world suffer from hypertensive disorders, which presents a significant risk to the general population's health. Therefore, the presence or absence of vitamin D might play a probable role in the development of preeclampsia and prenatal hypertension. However, a recent meta-analysis of clinical trials that evaluated vitamin D supplementation during pregnancy found no strong evidence of a protective effect on gestational hypertension (GH). These trials were conducted to evaluate whether or not vitamin D supplementation during pregnancy reduces the risk of developing GH. Therefore, the hypothesis is yet to be demonstrated differently.

There is a lot of evidence to suggest that vitamin D may moderate immunological responses. Because COVID-19 exacts such a heavy toll on the immune system, there has been a lot of interest in the possibility that vitamin D might mitigate or avoid adverse immunological responses. Vitamin D is capable of influencing several components of innate and adaptive immunity and might have the ability to affect the severity and consequences of COVID-19. Infections with SARS-CoV-2 lead to the downregulation of angiotensin-converting enzyme 2 (ACE2), which can result in a hazardous buildup of metabolites, leading to acute severe respiratory distress syndrome (ARDS), a feared complication of COVID-19. It has been discovered that vitamin D mitigates these interactions between SARS-CoV-2 and RAAS.

The vast majority of the available research indicates that SARS-CoV-2 infection prognosis and outcomes are better with sufficient concentrations of vitamin D, with or without supplementation. However, some studies indicate that there are no significant differences based on vitamin D levels and/or that there are no improvements following supplementation. Some people have even reported a lower rate of infection as a direct consequence of using the supplement in the past. In the context of the still ongoing COVID-19 pandemic, the current study aimed to determine whether vitamin D levels during pregnancy vary significantly among pregnant women who develop GH after SARS-CoV-2 infection.

RESULTS

At the study's conclusion, 144 pregnant women were found eligible for analysis. Anemia emerged as the most common comorbidity, affecting over 30% of the participants. This was closely followed by peripartum infections and gestational diabetes mellitus. Crucially, women with COVID-19 who did not experience gestational hypertension had a notably lower history of pregnancy-related complications, with percentages standing at 37.5% in the GH-CoV group and 25% in the GH group versus 22.9% for those without gestational hypertension.

Case-matching was executed based on comorbidity count and smoking status, revealing no significant discrepancies between the groups—around 70% had no comorbidities. Concerning COVID-19 vaccination, the data showed 25% of those in the GH-CoV group were vaccinated, contrasted with 37.5% in the CoV group and 29.2% in the GH

group. A significant 75% of those vaccinated received the Pfizer BNT162b2 vaccine. Notably, pregnant women who developed gestational hypertension post-COVID-19 contracted the virus much earlier in their pregnancy, specifically at a median of 9.4 weeks, compared to 14.8 weeks in the CoV group.

Although the vitamin D supplementation difference between the groups wasn't markedly significant, the CoV and GH-CoV groups showed a notable variance (33.3% vs. 47.9%; p-value = 0.038). A parallel difference was observed concerning calcium and magnesium supplementation between the CoV and GH groups. Pregnant women from the CoV group, who didn't develop gestational hypertension, had substantially higher vitamin D levels, with a median of 33.1 ng/mL, compared to 22.4 ng/mL in the GH-CoV group and 25.7 ng/mL in the GH group.

A detailed correlation analysis revealed a significant negative association between vitamin D levels and the patient's age. A crucial discovery was the negative correlation between serum 25(OH)D levels and systolic blood pressure. Interestingly, the week of SARS-CoV-2 infection correlated with the systolic blood pressure, as did the number of pregnancy-related comorbidities with the patient's age. Although a link was observed between vitamin D levels and systolic blood pressure, regression analysis found that the risk of gestational hypertension wasn't significantly higher among pregnant women with COVID-19 having insufficient or deficient vitamin D levels. Other pivotal risk factors included contracting SARS-CoV-2 during the first trimester and experiencing three or more pregnancies.

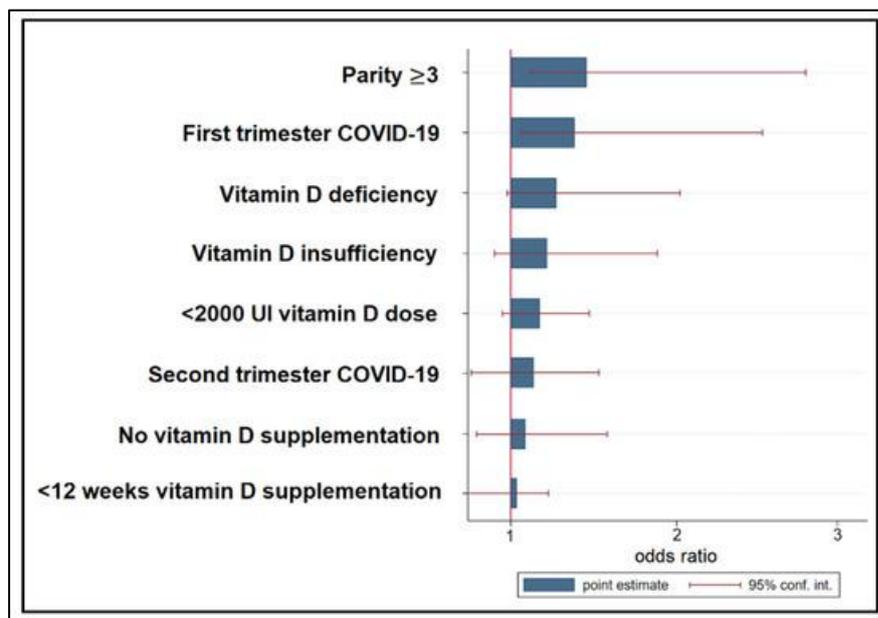


Figure 2 – Risk factor analysis for gestational hypertension.

CONCLUSIONS

It was observed that vitamin D deficiency and vitamin D insufficiency among pregnant women with COVID-19 may be an independent risk factor for the development of gestational hypertension. However, it can be hypothesized that the association between first-trimester SARS-CoV-2 infection and low vitamin D levels can influence the development of placental-mediated complications. Further research is required to investigate this association, including a larger sample and a healthy control group. Another question that arises is whether SARS-CoV-2 infection increases the risk of gestational hypertension as a primary mechanism, or whether hypertension could also be a risk factor for developing severe COVID-19.

STUDY 3: THE EFFECTS OF VITAMIN D SUPPLEMENTATION BEFORE 20 WEEKS OF GESTATION ON PREECLAMPSIA: A SYSTEMATIC REVIEW.

CONTEXT

Vitamin D, a fat-soluble vitamin that is essential for maintaining calcium and phosphorus homeostasis, has been postulated to play a role in the pathogenesis of preeclampsia. Vitamin D deficiency, commonly encountered in pregnant women due to inadequate dietary intake and reduced sun exposure, has been associated with an increased risk of preeclampsia. The immunomodulatory, anti-inflammatory, and vasculo-protective properties of vitamin D suggest that its supplementation during pregnancy may have potential benefits in preventing or attenuating the severity of preeclampsia.

Several observational and clinical studies have investigated the relationship between maternal vitamin D status and the risk of preeclampsia, yielding mixed results. While some studies have reported an inverse association between serum 25-hydroxyvitamin D (25-OH-D) levels and the risk of preeclampsia, others have found no significant relationship. Furthermore, the efficacy of vitamin D supplementation in early pregnancy as a preventive strategy for preeclampsia remains unclear, as randomized controlled trials have produced inconsistent findings.

A comprehensive synthesis of the available evidence is crucial for guiding clinical practice and informing public health policies. Systematic reviews and meta-analyses, which provide a rigorous and transparent approach to summarizing and appraising the existing literature, can help clarify the role of vitamin D supplementation in the prevention of preeclampsia. Our objective was to synthesize and critically appraise the available evidence from observational and interventional studies to determine the effects of early pregnancy vitamin D supplementation on the risk of preeclampsia. This review will provide a comprehensive and up-to-date summary of the current state of knowledge, contributing to a better understanding of the role of vitamin D in preeclampsia prevention and informing future research and clinical practice.

RESULTS

This systematic review analyzed a total of 1474 patients across five different studies. The demographic and clinical characteristics of the patients showed varied impacts concerning gestational age or Small for Gestational Age (SGA). For instance, in the study conducted by Sablok et al., there was a notable decrease in SGA among those in the vitamin D group (8.0%) compared to the control group (19.2%). In contrast, another study depicted only minor differences in SGA percentages between the two groups. Such differences hint at the possibility that the effects of vitamin D supplementation on gestational age or SGA might depend on various factors, like the dosage given, timing of the supplementation, or other unique variables specific to each study. Additionally, infant weights differed among studies. In one study, infants in the vitamin D group weighed more on average than those in the control group.

Vitamin D intake was not consistent across the studies. Dosages varied from a daily intake of 600 IU to a biweekly dose of 50,000 IU. For example, while some studies utilized daily supplementation, Sablock et al. opted for a biweekly regimen. This variability in dosing regimes could be a reason behind the inconsistent outcomes across the research. Furthermore, the time at which vitamin D supplementation commenced also varied among studies, with starting times ranging from the first trimester to as late as 20 weeks into the pregnancy. These discrepancies in supplementation initiation may have contributed to inconsistent results, emphasizing that the best time to start vitamin D supplementation for preventing preeclampsia is still uncertain.

Lastly, an evaluation of vitamin D levels and its association with preeclampsia risk revealed some vital findings. The definition of vitamin D insufficiency varied among studies, with some defining it as levels less than 20 ng/mL and others going up to 32 ng/mL. Cases, in general, showed lower vitamin D levels compared to controls, suggesting that low vitamin D levels might be linked to a heightened risk of preeclampsia. There was a broad prevalence of vitamin D insufficiency reported across studies, influenced possibly by differences in study populations or geographical locations. Additionally, in many studies, cases (those receiving vitamin D supplementation) generally demonstrated a lower prevalence of preeclampsia compared to controls, supporting the potential protective role of vitamin D supplementation. However, the observed effects of vitamin D supplementation on the severity of preeclampsia were mixed, with some studies showing a significant reduction in risk and others showing non-significant or even heightened risk.

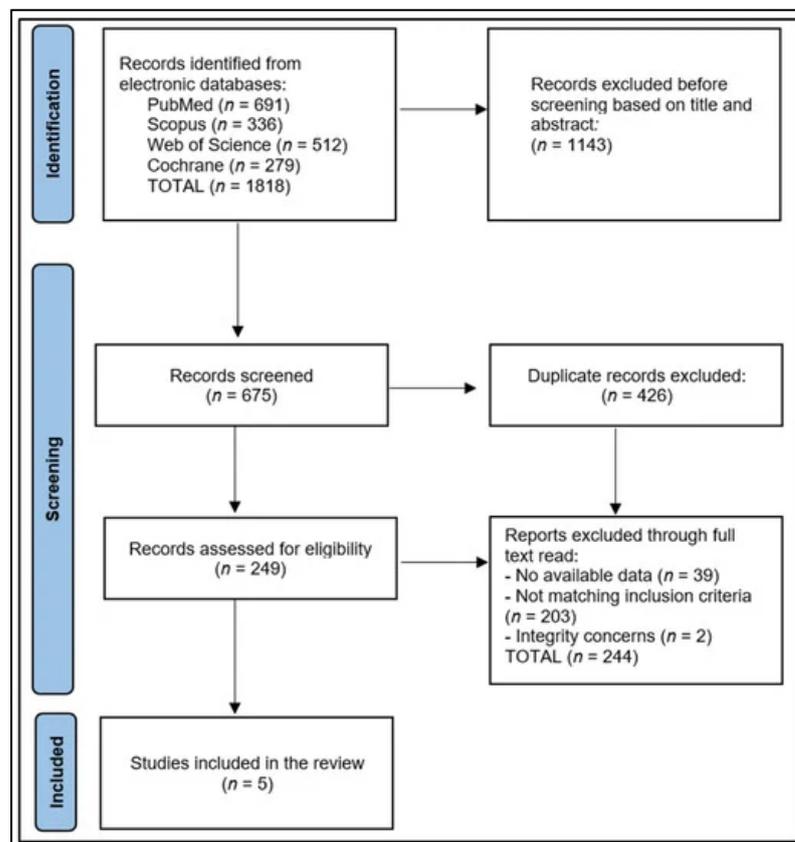


Figure 3 – PRISMA flow diagram.

CONCLUSIONS

The analysis of selected studies encompassing 1594 patients revealed mixed results, with some studies demonstrating a significantly reduced preeclampsia risk associated with vitamin D supplementation while others showed no significant difference. The discrepancies in outcomes may be attributed to variations in vitamin D dosages, the timing of supplementation, baseline vitamin D levels, sunlight exposure, and other study-specific factors. Moreover, inconsistencies in defining vitamin D insufficiency and diverse secondary outcomes were observed across the studies. Although the current evidence suggests a potential role for vitamin D supplementation in reducing the risk of preeclampsia, further research is needed to identify the optimal dosage and timing of supplementation for preeclampsia prevention or attenuation.