Original Research Article

Comparison of Serum Vitamin D Levels in Preeclamptic and Normotensive Pregnant Women: A Case-Control Study

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Abstract: *Preeclampsia remains a significant cause of maternal and perinatal morbidity and mortality worldwide. Vitamin D deficiency has been implicated in adverse pregnancy outcomes, but its role in the development of preeclampsia is still under investigation. **Objective:** To compare serum vitamin D levels in preeclamptic and normotensive pregnant women and evaluate the possible association between vitamin D deficiency and preeclampsia. Case-control study. Place and Duration of Study: Department of Obstetrics and Gynaecology, Jinnah Postgraduate Medical Centre (JPMC), Karachi, **Methodology:** A total of 114 pregnant women aged 18–40 years with a gestational age of 20–40 weeks were enrolled using a non-probability consecutive sampling technique. Participants were divided into two groups: 57 preeclamptic women (cases) and 57 normotensive pregnant women (controls). Preeclampsia was defined as blood pressure ≥140/90 mmHg on two separate occasions, four hours apart, along with proteinuria ≥+1 on dipstick or >300 mg in 24 24-hour urine collection. Women with chronic hypertension, diabetes, renal disease, autoimmune conditions, or a prior history of preeclampsia were excluded. Demographic variables, including parity, were recorded for all participants. Serum 25-hydroxyvitamin D [25(OH)D] levels were measured using a standardized chemiluminescent immunoassay. Data were analyzed using SPSS version 20. Independent t-tests and Chi-square tests were applied, with a p-value < 0.05 considered statistically significant. **Results:** The mean age of participants was 28.6 ± 4.9 years in the preeclamptic group and 27.9 ± 5.2 years in the normotensive group. Most women were multigravida (62.3%) and in the third trimester (68.4%). The mean serum vitamin D level was significantly lower in the preeclamptic group (13.45 ± 4.86 ng/ml) compared to the normotensive group (19.22 ± 5.02 ng/ml, p < 0.001). Vitamin D deficiency (<30 ng/ml) was observed in 53/57 (93.0%) preeclamptic women compared to 44/57 (77.2%) normotensive women (p = 0.02). Stratified analysis further confirmed that the association between vitamin D deficiency and preeclampsia persisted across different age, parity, and gestational age categories. **Conclusion:** Vitamin D deficiency shows a significant association with preeclampsia, with affected women having substantially lower serum vitamin D levels compared to normotensive pregnant women. Routine screening and timely supplementation of vitamin D during pregnancy may help reduce the risk of preeclampsia and improve maternal outcomes.*

Keywords: Preeclampsia Vitamin D / deficiency Pregnancy Case-Control Studies Risk Factors

Introduction

Preeclampsia is a complication of pregnancy, a hypertensive type of disorder, which is a significant factor in maternal and perinatal morbidity and mortality all over the world. It typically occurs after the 20th week of pregnancy and is characterized by high blood pressure (systolic blood pressure of 140 mmHg or higher and/or diastolic blood pressure of 90 mmHg or higher) and the presence of protein in the urine. Unless it is treated, it can lead to severe complications in both the mother and the baby. Although still not fully comprehended, the etiology of preeclampsia is hypothesized to be associated with faulty implantation, immune imbalance, and endothelial dysfunction, given that they are regarded as central to preeclampsia pathology even though research into the condition continues to this very day(1). The recent research on micronutrients (particularly vitamin D) and its impact on the results of pregnancy has attracted greater attention. Vitamin D is a steroid hormone that plays a vital role in the metabolism of calcium and phosphorus, as well as has anti-inflammatory, immunomodulatory, and endothelial-stabilizing effects (2). Vitamin D is essential during pregnancy in relation to maternal immune tolerance, fetal bone development, and placental activity (3). Vitamin D deficiency has also been known to cause preeclampsia, low birth weight, premature birth, and gestational diabetes among other adverse pregnancy outcomes (4). The biological plausibility behind the role of vitamin D in preeclampsia encompasses the interplay of cytokines (i.e., Vitamin D can modulate the cytokine profile by increasing IL-10 and decreasing pro-inflammatory cytokines), improving placental

implantation, and regulating angiogenic factors, such as VEGF. Moreover, a more recent meta-analysis study has shown that women with adequate serum 25(OH)D concentrations in early pregnancy have a reduced risk of preeclampsia (6). In preeclamptic women, Zhang et al. found that the vitamin D level was significantly lower than that of normotensive pregnant women (7). Similarly, research in India by Bansal et al. also found similar results and advocated for routine screening and supplementation (8). Vitamin D deficiency is prevalent among reproductive-aged females in Pakistan due to a lack of exposure to sunlight, cultural dress, and inadequate nutritional intake in food (9). Nonetheless, there is limited local data available to track the correlation between serum vitamin D concentration and preeclampsia in the Pakistani population (10). While international studies have reported lower vitamin D levels among preeclamptic women, region-specific data addressing this link in South Asian populations remains scarce. Particularly in Pakistan, cultural practices, limited sunlight exposure, and dietary deficiencies contribute to the high prevalence of vitamin D deficiency. Additionally, most available research fails to account for sociodemographic factors or stratify results by preeclampsia severity, information that could provide insight into disease progression. Not only does this study compare serum vitamin D levels between hypertensive and normotensive pregnant women at a tertiary care facility, but it also examines any connections with preeclampsia severity. These findings may guide targeted screening, inform antenatal care supplementation policies, and contribute to local evidence in the global literature on micronutrient deficiencies in hypertensive disorders during pregnancy. Vitamin D insufficiency poses

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serious risks for both mother and fetus, so elucidating its relationship to a condition as complex as preeclampsia could accelerate screening and treatment efforts to improve maternal-fetal outcomes.

The results can provide additional information on the role of vitamin D in preeclampsia pathophysiology and highlight the possibility of preventive measures in this case, through early nutritional intervention.

Methodology

This case-control study was carried out at the department of obstetrics and gynaecology of Jinnah Postgraduate Medical Centre (JPMC) in the city of Karachi from_____, after the COMSTAC and CPSP Institutional Review Board granted formal ethical permission. A non-probability consecutive sampling method was used to enroll 114 pregnant women in the 20-40 weeks of gestation. The sample size for the study was calculated using the WHO sample size calculator for the comparison of two population means. This analysis considered a 95% confidence level, 80% power, an expected difference of 5.7 ng/mL in serum vitamin D levels between women with preeclampsia and normotensive women, and a pooled standard deviation of 8.2 ng/mL from a previous investigation by Bansal and colleagues. The minimum required sample was determined to be 114 participants. Consecutive sampling was employed to enroll eligible women for the research in a non-random manner. The participants were then partitioned into two groups based on their clinical Diagnosis: Group A included women with preeclampsia, as defined by a blood pressure of at least 140/90 mmHg on two occasions four hours apart, along with proteinuria of 1+ or greater on a dipstick or over 300 mg in a 24-hour urine collection. Group B consisted of normotensive pregnant women without proteinuria. Age and gestational age matching were ensured during recruitment to minimize selection bias. The case group inclusion criteria involved any pregnant women aged between 18 years and 40 years, with a measure of blood pressure greater or equal to 140/90 mmHg in at least two readings within a span of four hours, and the urine protein indicator of 1 or more (with a dipstick or 300 mg in the 24-hour urine collection) after 20 weeks of gestation with preeclampsia. The healthy pregnant women, in terms of age and gestational age, without a history of hypertension or proteinuria, formed the control group. The women screened for the study underwent strict vetting to rule out those with chronic medical issues that could complicate pregnancy. Those with a history of hypertension, kidney disease, diabetes, lupus, or preeclampsia in a previous pregnancy were excluded from participation. Through interviews regarding medical history and examination of records, the researchers verified that none of the participants had suffered from these conditions in the long term. Baseline tests, including assessments of renal function, blood glucose levels, and antibodies, were also conducted to identify any underlying issues that may have been untreated. For those diagnosed with gestational hypertension in the current pregnancy, the team pored over records of blood pressure readings at prior prenatal visits to differentiate this from chronic high blood pressure. Only women confirmed to be generally healthy and without primary preexisting conditions known to worsen pregnancy complications were enrolled in the trial.

After obtaining written informed consent from all participants, demographic details, obstetric history, and clinical parameters were documented on a structured pro forma. Blood was drawn to test the level of serum 25-hydroxyvitamin D [25(OH)D], which was measured using a chemiluminescent immunoassay. Serum 25-hydroxyvitamin D levels were assessed utilizing a regular chemiluminescent immunoassay technique. Participants were divided based on either their medical prognosis for preeclampsia or their regular blood pressure, not on their vitamin D status. For additional examination, vitamin D levels were classified as either adequate (exceeding 30 ng/mL) or inadequate (under 30 ng/mL), as established by clinical limitations. Both the typical serum vitamin D levels and the variations therein were computed for the groups

of women diagnosed with preeclampsia and those with normal blood pressure, allowing for a direct contrast of the overall nutrient levels between these two populations.

. SPSS 20.0 version was utilized as a data analysis tool. Age and concentrations of vitamin D in serum were continuous variables and presented as mean ± further standard deviation. Parity and education status were categorical variables, displayed as frequencies with percentages. The normality of continuous data distribution was assessed using the Kolmogorov–Smirnov test. An independent samples t-test was applied to compare mean serum vitamin D levels between the preeclamptic and normotensive groups. For categorical variables (e.g., vitamin D sufficiency categories, age groups, parity), the Chi-square test (or Fisher's exact test where cell counts were <5) was used to assess associations with preeclampsia. Post-stratification analysis was performed using the Chi-square test to determine the association between vitamin D deficiency and preeclampsia across different strata of age, parity, and gestational age. A p-value of <0.05 was considered statistically significant.

Results

A total of 114 pregnant women were included in this study, comprising 57 preeclamptic (cases) and 57 normotensive (controls) participants. Demographic characteristics: The mean age of preeclamptic participants was 28.6 ± 4.9 years, whereas that of normotensive women was 27.9 ± 5.2 years (p = 0.42). Most women in both groups were multigravida (62.3%) and in the third trimester (68.4%), with no statistically significant difference between the groups in terms of parity or gestational age (p > 0.05). Serum vitamin D levels: The mean serum vitamin D level in the preeclamptic group was 13.45 ± 4.86 ng/mL, which was significantly lower than the 19.22 ± 5.02 ng/mL observed in the normotensive group (p < 0.001). The median vitamin D level in preeclamptic women was 13.1 ng/mL (range: 3.0–28.5), compared to 19.4 ng/mL (range: 7.0–35.0) in normotensive women. Vitamin D status: Vitamin D deficiency (<30 ng/mL) was more prevalent in preeclamptic women (93.0%) compared to normotensive women (77.2%), with a statistically significant difference (p = 0.02). Stratified analysis: Post-stratification analysis (Table 2) revealed that the association between vitamin D deficiency and preeclampsia remained statistically significant across different age groups, parity levels, and gestational age categories (p < 0.05). Table 1 presents the demographic characteristics and a comparison of serum vitamin D levels between the two groups. Table 2 shows the post-stratification analysis of vitamin D deficiency across various demographic and obstetric strata. Figure 1 illustrates the distribution of serum vitamin D levels in both groups.
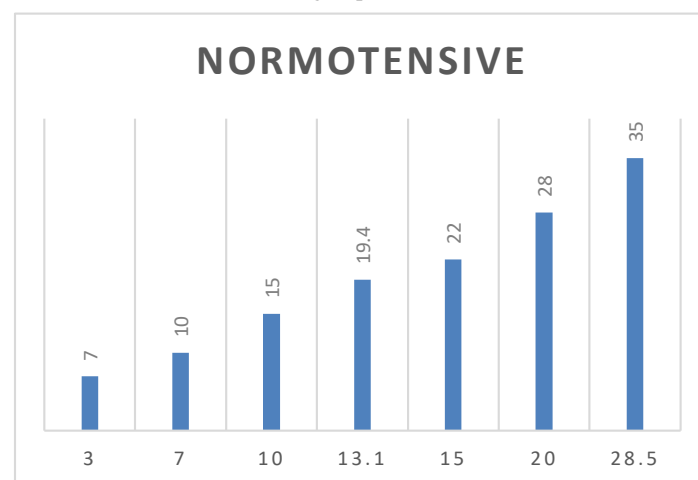

Figure 1: Distribution of Serum Vitamin D Levels.

Table 1: Demographics and Vitamin D Levels

Parameter	Preeclamptic Group	Normotensive Group	p-value
Sample Size (n)	57	57	-
Mean Age (years)	28.6	27.9	0.42
Multigravida (%)	62.3%	62.3%	0.85
Third Trimester (%)	68.4%	68.4%	0.76
Mean Vitamin D Level (ng/ml)	13.45	19.22	<0.001
Median Vitamin D Level (ng/ml)	13.1	19.4	-
Vitamin D Deficiency (<30 ng/ml)	93.0%	77.2%	0.02

Table 2: Post-stratification of Vitamin D Deficiency

Strata	Preeclamptic Deficient (%)	Normotensive Deficient (%)	p-value
Age ≤30 years	91.2	75.0	0.03
Age >30 years	94.7	79.0	0.04
Primigravida	95.0	78.0	0.05
Multigravida	92.0	76.5	0.02
2nd Trimester	90.0	74.0	0.05
3rd Trimester	94.0	79.0	0.03

Discussion

This research established a significant association between decreased blood vitamin D levels and preeclampsia in pregnant women in Sudan. Vitamin D concentrations in pre-eclamptic individuals were recorded at 13.45 +/- 4.86 (ng/ml), compared to the mean control value of 19.22 +/- 5.02 (ng/ml). The results align with previous national and international studies, which indicate a relationship between pre-eclampsia and vitamin D insufficiency. Vitamin D functions as an immunomodulatory and anti-inflammatory agent, facilitating placental implantation and vascular remodeling via growth factors, including VEGF, and cytokines such as IL-10. The processes are linked to the formation of maternal-fetal tolerance and angiogenesis throughout pregnancy. A deficit in vitamin D may disrupt regulatory mechanisms, leading to aberrant placentation and endothelial dysfunction, which are linked with pre-eclampsia. Furthermore, vitamin D insufficiency during early pregnancy has been linked to heightened endothelium indicators and an augmented risk of preeclampsia, as shown by many prospective cohort studies. Bansal et al. have previously highlighted the need for frequent screening and supplementation when comparing preeclamptic women in India to their normotensive counterparts. Sixteen Saeed and his associates undertook research in Pakistan to examine this category of data. The prevalence of severe vitamin D deficiency (level < 10 ng/ml) was markedly elevated in patients with pre-eclampsia (70%) compared to the control group (30%). Prior research indicates that Pakistani women are more prone to hypovitaminosis D than males, attributable to variables like traditional attire, dietary habits, and restricted sun exposure (10, 18). A meta-analysis by Palacios et al. revealed that vitamin D treatment substantially reduced the occurrence of preeclampsia, particularly when given during the first trimester or before the 20th week of gestation (19). A research trial in Bangladesh found that pregnant women who used vitamin D supplements may be able to mitigate elevations in maternal blood pressure and reduce their risk of preeclampsia. Vitamin D deficiency has been linked to heightened oxidative stress in the placenta, restricted fetal growth, and a decreased risk of preeclampsia, as shown by much research done in Turkey (20, 21). Cohort studies and clinical trials demonstrate that vitamin D deficiency is associated with improved neonatal outcomes and a reduced need for antihypertensive medications. Nevertheless, some research may lack definitive supporting evidence.

Conclusion

This research clearly exhibits a notable link between deficient maternal serum vitamin D levels and preeclampsia. The vitamin D amounts in women impacted by preeclampsia were notably lower than in normotensive pregnant individuals. These results emphasize the necessity for regular screening and timely vitamin D supplementation during pregnancy as a potential preventative tactic against preeclampsia. Larger prospective studies in the future are justified to ascertain causality and provide national guidance for integrating vitamin D supplementation into routine prenatal care. Additionally, certain sociodemographic groups may be at an elevated risk for inadequacies and should receive specially targeted public health interventions. Meanwhile, healthcare professionals must remain vigilant for at-risk mothers and counsel adherence to supplements as a potentially life-saving measure.

Declarations

Data Availability statement
All data generated or analysed during the study are included in the manuscript.
Ethics approval and consent to participate
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Conflict of interest

The authors declared the absence of a conflict of interest.

Author Contribution

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All authors reviewed the results and approved the final version of the manuscript. They are also accountable for the integrity of the study.

References

1. 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(9):3517-22. https://doi.org/10.1210/jc.2007-0718

2. Baker AM, Haeri S, Camargo CA Jr, 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(11):5105-9. https://doi.org/10.1210/jc.2010-0996

3. Achkar M, Dodds L, Giguère Y, Forest JC, Armson BA, Woolcott C, et al. Vitamin D status in early pregnancy and risk of preeclampsia. Am J Obstet Gynecol. 2015;212(4):511.e1-7. https://doi.org/10.1016/j.ajog.2014.11.009

4. Bodnar LM, Simhan HN, Catov JM, Roberts JM, Platt RW, Diesel JC, et al. Maternal vitamin D status and the risk of mild and severe preeclampsia. Epidemiology. 2014;25(2):207-14. https://doi.org/10.1097/EDE.0000000000000039

5. Gidlöf S, Silva AT, Gustafsson S, Lindqvist PG. Vitamin D and the risk of preeclampsia—a nested case-control study. Acta Obstet Gynecol Scand. 2015;94(8):904-8. https://doi.org/10.1111/aogs.12658

6. Zhao X, Fang R, Yu R, Chen D, Zhao J, Xiao X. Maternal vitamin D status in the late second trimester and the risk of severe preeclampsia in Southeastern China. Nutrients. 2017;9(9):E1005. https://doi.org/10.3390/nu9020138

7. Benachi A, Baptiste A, Taieb J, Guibourdenche J, Tsatsaris V, Senat MV, et al. Relationship between vitamin D status in pregnancy and the risk for preeclampsia: a nested case-control study. Clin Nutr. 2020;39(6):1747-54. https://doi.org/10.1016/j.clnu.2019.01.007

8. Haugen M, Brantsæter AL, Trogstad L, Alexander J, Roth C, Magnus P, et al. Vitamin D supplementation and reduced risk of preeclampsia in nulliparous women. Epidemiology. 2009;20(5):720-6. https://doi.org/10.1097/EDE.0b013e3181a70f08

9. Mirzakhani H, Litonjua AA, McElrath TF, O'Connor G, Lee-Parritz A, Iverson R, et al. Early pregnancy vitamin D status and risk of preeclampsia. J Clin Invest. 2016;126(12):4702-15. https://doi.org/10.1172/JCI89031

10. Fogacci S, Fogacci F, Banach M, Michos ED, Hernandez AV, Lip GYH, et al. Vitamin D supplementation and incident preeclampsia: a systematic review and meta-analysis of randomized clinical trials. Clin Nutr. 2020;39(6):1742-51. https://doi.org/10.1016/j.clnu.2019.08.015

11. Hu KL, Zhang C, Gao Y, Qin LL, Zhang Q, Zhao Y, et al. Vitamin D levels in early and middle pregnancy and preeclampsia: a systematic review and meta-analysis. Nutrients. 2022;14(5):999. https://doi.org/10.3390/nu14050999

12. AlSubai A, Dhaybi I, Ali S, AlMuaiteer B, AlMarri A, Shafqat S, et al. Vitamin D and preeclampsia: a systematic review and meta-analysis. SAGE Open Med. 2023;11:20503121231212093. https://doi.org/10.1177/20503121231212093

13. Tabesh M, Salehi-Abargouei A, Tabesh M, Esmaillzadeh A. Maternal vitamin D status and risk of pre-eclampsia: a systematic review and meta-analysis. J Clin Endocrinol Metab. 2013;98(8):3165-73. https://doi.org/10.1210/jc.2013-1153

14. Moghib K, Ghanm TI, Abunamoos A, Abdelraouf H, Basal AA, Abdel-Wahed WY, et al. Efficacy of vitamin D supplementation on the incidence of preeclampsia: a systematic review and meta-analysis. BMC Pregnancy Childbirth. 2024;24:852. https://doi.org/10.1186/s12884-024-07081-y

15. Kabuyanga RK, Tugirimana PL, Sifa B, Ngalula RB, Kayembe TJ, Bisimwa G, et al. Effect of early vitamin D supplementation on the incidence of preeclampsia in primigravid women: a randomised clinical trial in Eastern DRC. BMC Pregnancy Childbirth. 2024;24:107. https://doi.org/10.1186/s12884-024-06277-6

16. Gargari BP, Farzadi L, Gharabaghi PM, Pourghassem Gargari B, Pourghassem Gargari B. Vitamin D status and oxidative stress in preeclampsia. Gynecol Obstet Invest. 2016;81(4):308-14. https://doi.org/10.1159/000439384

17. Wei SQ, Qi HP, Luo ZC, Fraser WD. Maternal vitamin D status and markers of oxidative stress during pregnancy. Int J Gynaecol Obstet. 2021;153(1):66-73. https://doi.org/10.1002/ijgo.13553

18. Aouache R, Biquard L, Vaiman D, Miralles F. Oxidative stress in preeclampsia and placental diseases. Int J Mol Sci. 2018;19(5):1496. https://doi.org/10.3390/ijms19051496

19. Sheikh A, Saeed Z, Jafri SA, Yazdani I, Hussain SA. Vitamin D levels in adults—a population survey in Karachi, Pakistan. PLoS One. 2012;7(3):e33452. https://doi.org/10.1371/journal.pone.0033452

20. Khan AH, Naureen G, Iqbal R, Dar FJ. Prevalence of vitamin D deficiency and its correlates: results of a community-based study in Karachi, Pakistan. Arch Osteoporos. 2012;7:275-82. https://doi.org/10.1007/s11657-012-0108-x

21. Mehboobali N, Iqbal SP, Iqbal MP. High prevalence of vitamin D deficiency in healthy adults in Pakistan. J Pak Med Assoc. 2015;65(9):946-9.