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Association between maternal vitamin D levels and the risk of gestational diabetes mellitus: A case-control study.

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Abstract

Background
Vitamin D deficiency has emerged as a potential risk factor for gestational diabetes mellitus (GDM), supported by evidence of its role in insulin sensitivity and glucose metabolism.
Objectives: To compare serum 25-hydroxyvitamin D [25(OH)D] levels between pregnant women with and without GDM, and to assess the association between vitamin D status and glycemic control.

Methods
A hospital-based case-control observational study was conducted in the Department of Obstetrics and Gynaecology, Narayan Medical College, Sasaram, from December 2023 to December 2024. The study included 200 third-trimester pregnant women—100 with GDM (Group A) and 100 with normal glucose tolerance (Group B). Demographic, lifestyle, and biochemical data were collected. Serum 25(OH)D and HbA1c levels were measured using chemiluminescence immunoassay. Statistical analysis included t-tests and chi-square tests, with p<0.05 considered significant.

Results
Mean serum vitamin D levels were significantly lower in the GDM group (10.15 ± 5.85 ng/mL) than in controls (19.22 ± 7.34 ng/mL; p<0.001). Vitamin D deficiency was more prevalent among GDM cases (90.0%) compared to controls (60.0%; p<0.001). HbA1c and fasting blood glucose levels were significantly higher in GDM patients (p<0.001). An inverse correlation was observed between serum vitamin D and HbA1c. No significant differences were noted in maternal age, diet, or family history of diabetes (p= 0.21, 0.63, 0.60), whereas prior GDM and low sunlight exposure were significantly associated with GDM (p<0.01)

Conclusion
Low maternal serum vitamin D levels are significantly associated with GDM and poor glycemic control, indicating a potential role in its pathogenesis.

Recommendations
Routine screening of serum 25(OH)D levels during antenatal care is advised, particularly for women at high risk of GDM. Vitamin D supplementation under medical supervision may help reduce GDM risk and improve glycemic outcomes.

Keywords: *Gestational diabetes mellitus, Vitamin D, 25-hydroxyvitamin D, Glycated Hemoglobin, pregnancy, Insulin resistance, Glycemic control*
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Introduction

Gestational Diabetes Mellitus (GDM) is defined as glucose intolerance of varying severity, with onset or first recognition during pregnancy. It is one of the most common metabolic complications of pregnancy, with a global prevalence ranging from 7% to 14%. The incidence of GDM is rising, primarily due to factors such as increasing maternal age, higher rates of obesity, and sedentary lifestyles. GDM is associated with significant short- and long-term health risks for both the mother and fetus, including

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preeclampsia, cesarean delivery, macrosomia, neonatal hypoglycemia, and an elevated risk of developing type 2 diabetes mellitus later in life for both mother and child.[12]

In recent years, attention has turned to the role of micronutrients—particularly vitamin D—in modulating pregnancy outcomes. Vitamin D exists in two biologically relevant forms: vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol), which are synthesized in the skin upon exposure to ultraviolet B radiation or obtained from dietary sources. Following hepatic conversion to 25-hydroxyvitamin D [25(OH)D], the major circulating form and clinical marker of vitamin D status, it is further hydroxylated in the kidneys to its active form, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D [34]

Although traditionally recognized for its role in calcium and phosphate homeostasis, vitamin D has been increasingly implicated in non-skeletal physiological functions. Vitamin D receptors (VDRs) are widely expressed in various tissues, including pancreatic β-cells, adipose tissue, and the placenta, suggesting their involvement in glucose metabolism, insulin secretion, and insulin sensitivity.[5] Animal studies and human observational research have indicated that adequate vitamin D levels may enhance β-cell function and reduce insulin resistance, two key pathophysiological mechanisms in GDM development.[6]

Furthermore, vitamin D is known to exert immunomodulatory effects, including suppression of pro-inflammatory cytokines such as TNF-α and IL-2, both of which have been implicated in the pathogenesis of GDM. It also promotes insulin receptor expression and facilitates glucose uptake in peripheral tissues.[7] Despite these biological mechanisms, clinical trial data remain inconclusive. While observational studies suggest an inverse relationship between maternal vitamin D deficiency and GDM, interventional studies have yielded inconsistent results regarding the efficacy of vitamin D supplementation in preventing or managing GDM. Heterogeneity in study findings may be attributed to confounding variables such as differences in baseline vitamin D levels, genetic predisposition, sun exposure, dietary intake, body mass index, and ethnicity. Additionally, during pregnancy, physiological elevations in circulating 1,25$(OH)_2$D—partly driven by placental production—suggest a unique adaptive role of vitamin D in supporting maternal and fetal health.[8]

Given its modifiable nature, cost-effectiveness, and the public health burden of GDM, exploring the association between maternal vitamin D status and GDM risk remains a critical area of research.

Formulas

Clarifying this relationship may inform antenatal care guidelines and nutritional strategies aimed at improving maternal and neonatal outcomes.[9]

Aim

- To assess the correlation between maternal serum vitamin D levels and the occurrence of Gestational Diabetes Mellitus (GDM) in pregnant women.

Objectives

- To estimate the serum 25-hydroxyvitamin D \[25(OH)D] levels in pregnant women during the second trimester.
- To evaluate the statistical association between serum vitamin D levels and the incidence of GDM.
- To compare maternal and neonatal outcomes in GDM patients with sufficient versus deficient vitamin D status.

Materials and methods

Study setting

Narayan Medical College & Hospital (NMCH), Sasaram, established in 2008, is a recognized private medical institution affiliated with Veer Kunwar Singh University. It offers MBBS and MD/MS programs, supported by a 1000+ bedded teaching hospital and modern facilities, serving the healthcare needs of Bihar and Jharkhand.

Type and duration of study

This was a hospital-based case-control observational study, conducted over a period of 12 months from December 2023 to December 2024.

Sample size

Assuming that the serum vitamin D level in GDM and non-GDM can be one of the comparison criteria. As per the previous study the mean serum vit D level was 15.51 ± 8.33 vs. 24.67 ± 6.26 (based on study by Mustafa Rabiee Ali et al), at 2-sided test with 95% confidence level (α=5%) and 80% power, expected sample size in both group is 10 each, i.e total 20, but to increase the power of study sample size 100 in each group were taken, so total 200 sample size was taken.

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This calculator uses the following formulas to compute sample size.

$$n_1 = \frac{(\sigma_1^2 + \sigma_2^2 / \kappa)(z_{1-\alpha/2} + z_{1-\beta})^2}{\Delta^2}$$

Efforts to address potential sources of bias

To minimize bias, cases and controls were uniformly selected from the same hospital and period.

Inclusion criteria

- Pregnant women in the third trimester with an established diagnosis of Gestational Diabetes Mellitus (GDM) as per DIPSI guidelines.
- Pregnant women with normal blood sugar levels are attending ANC during the same period.

Exclusion criteria

- History of pre-existing type 1 or type 2 diabetes mellitus.
- History of chronic medical conditions such as hypertension, cardiac disease, or other systemic disorders.
- Pregnancy-related complications affecting maternal or fetal outcomes.

Data collection procedure

A total of 200 pregnant women participated in the study, comprising 100 pregnant women diagnosed with Gestational Diabetes Mellitus (GDM) (Group A – Cases) and 100 age-matched pregnant women with normal glucose tolerance (Group B – Controls). The participants were recruited from those attending the antenatal care clinic at Narayan Medical College, Sasaram. A comprehensive and structured history was obtained from each participant, with particular emphasis on demographic and obstetric parameters including maternal age, parity, gestational age at diagnosis of GDM, family history of diabetes mellitus, and previous history of GDM in prior pregnancies.

Standardized assays with blinded lab staff reduced measurement bias, while structured tools minimized recall and information bias. Confounders like age, BMI, diet, and family history were documented and analyzed. Consistent definitions of vitamin D status ensured validity.

Additionally, detailed information regarding insulin use among GDM cases was meticulously recorded, noting specifics such as the type of insulin administered (short-acting, intermediate-acting, or long-acting), dosage regimen, and frequency of administration. Each participant underwent a thorough physical evaluation that included a general physical examination assessing vital parameters and anthropometric measurements, systemic examination for any existing medical conditions, and detailed obstetric examination to monitor fetal well-being and pregnancy progression.

Body Mass Index (BMI) was calculated for all participants using standard anthropometric measurements of height and weight, ensuring that the BMI distribution was matched between cases and controls to reduce potential confounding effects. This rigorous approach allowed for a robust comparison between the groups regarding serum vitamin D status and its association with gestational diabetes mellitus.

Laboratory investigations:

Blood samples were systematically collected from all study participants after obtaining informed written consent. Venous blood samples were drawn under aseptic precautions, preferably in the morning hours, and immediately transferred to sterile, appropriately labeled collection tubes. The collected samples were then promptly transported to the laboratory under controlled conditions for biochemical analysis.

Serum 25-hydroxyvitamin D \[25(OH)D] concentrations were measured using the Chemiluminescence Immunoassay (CLIA) method, a highly sensitive and reliable laboratory technique commonly employed to quantify vitamin D status.

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Serum samples underwent processing in an automated immunoassay analyzer, and vitamin D levels were reported in nanograms per milliliter (ng/mL). Based on the obtained serum 25(OH)D concentrations, the vitamin D status of participants was carefully categorized into three clinically relevant groups:

Vitamin D deficiency: serum 25(OH)D levels less than 20 ng/mL
Vitamin D insufficiency: serum 25(OH)D levels between 20 and 30 ng/mL
Vitamin D sufficiency: serum 25(OH)D levels greater than 30 ng/mL

Additionally, to evaluate glycemic control among pregnant women diagnosed with Gestational Diabetes Mellitus (GDM), glycosylated hemoglobin (HbA1c) levels were also assessed. HbA1c measurements were performed utilizing the chemiluminescence assay method, an advanced, precise laboratory technique that measures the proportion of hemoglobin molecules bound to glucose. HbA1c provided an accurate reflection of average glycemic status over the preceding 2–3 months, thereby serving as a reliable biochemical marker to gauge glucose management in the GDM participants. These comprehensive biochemical assessments facilitated an in-depth comparative analysis of maternal vitamin D status and glycemic control between GDM cases and non-diabetic controls.

Statistical analysis

Collected data were analyzed using appropriate statistical software (such as SPSS). Continuous variables, including serum vitamin D and HbA1c levels, BMI, and maternal age, were expressed as means and standard deviations (SD). Categorical variables, such as vitamin D status categories (deficiency, insufficiency, sufficiency) and parity, were presented as frequencies and percentages. The association between vitamin D status and GDM was assessed using the Chi-square test for categorical variables and the independent t-test for continuous variables. A p-value < 0.05 was considered statistically significant.

Results

Participant flow

Potentially eligible participants (n=250)

Examined for eligibility (n=220)

Excluded (n=20)
- Not eligible: 12
- Declined: 8

Eligible and consented (n=200)

Group A (GDM cases) n=100

Group B (Controls) n=100

Completed analysis (n=200)

Table 1: Demographic characteristics of study participants

Variables	Group A (Cases, n=100)	Group B (Controls, n=100)	p-value
Maternal Age (years), Mean±SD	27.34 ± 6.44	26.22 ± 6.34	0.21(NS)
Vegetarian Diet (%)	11.5%	9.5%	0.63(NS)
Non-Vegetarian Diet (%)	88.5%	90.5%	0.65(NS)
Past H/O GDM (%)	9.0%	1.0%	<0.01(S)
Family H/O Type 2 DM (%)	9.5%	7.0%	0.60(NS)

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The demographic characteristics of the study participants are summarized in Table 1. The mean maternal age was comparable between the two groups, with cases (women diagnosed with GDM) having a mean age of 27.34 ± 6.44 years, and controls (normal pregnant women) having a mean age of 26.22 ± 6.34 years; this difference was not statistically significant (p > 0.05). Dietary habits were also similar between the groups, with a vegetarian diet being slightly higher among cases (11.5%) compared to controls (9.5%), and a non-vegetarian diet prevalent in both groups (88.5% in cases and 90.5% in controls); these differences were statistically nonsignificant (p > 0.05). A notable and statistically significant difference (p < 0.01) was observed regarding a history of gestational diabetes mellitus, which was significantly higher in cases (9.0%) compared to controls (1.0%). However, family history of type 2 diabetes mellitus showed no significant difference between groups (cases: 9.5%; controls: 7.0%; p > 0.05).

Table 2: Lifestyle and dietary factors in study groups

Variables	Group A (Cases, n=100)	Group B (Controls, n=100)	p-value
Adequate Calcium Intake (%)	63.0%	71.0%	>0.05(NS)
Periconceptional Multivitamin Intake (%)	26.0%	31.0%	>0.05(NS)
Physical Activity in Sunlight (>3 hrs/day)	8.0%	32.0%	<0.01(S)

Table 2 summarizes lifestyle and dietary factors among the study participants. The proportion of women with adequate calcium intake was slightly lower among cases (63.0%) compared to controls (71.0%); however, this difference was not statistically significant (p > 0.05). Similarly, periconceptional multivitamin intake was observed in 26.0% of cases and 31.0% of controls, showing no statistically significant difference between the groups (p > 0.05). In contrast, a statistically significant difference (p < 0.01) was noted regarding physical activity in sunlight (>3 hours/day), with a notably lower proportion of cases (8.0%) engaging in sufficient sunlight exposure compared to controls (32.0%). This indicates that decreased sunlight exposure may be an important associated factor for lower vitamin D levels in women diagnosed with GDM.

Table 3: Biochemical parameters of study groups

Variables	Group A (Cases, n=100)	Group B (Controls, n=100)	p-value
Fasting Blood Sugar (mg/dL), Mean ± SD	95.34 ± 6.98	79.42 ± 4.67	<0.001(S)
HbA1c (%), Mean ± SD	6.72 ± 1.05	5.14 ± 0.28	<0.001(S)
Vitamin D Level (ng/mL), Mean ± SD	10.15 ± 5.85	19.22 ± 7.34	<0.001(S)
Vitamin D Deficiency (%)	90.0%	60.0%	<0.001(S)

Table 3 illustrates the biochemical parameters of the study participants, highlighting significant differences between cases (GDM group) and controls. The mean fasting blood sugar was significantly higher in cases (95.34 ± 6.98 mg/dL) compared to controls (79.42 ± 4.67 mg/dL), with a highly significant p-value (<0.001). Glycosylated hemoglobin (HbA1c), a marker of glycemic control, was also markedly elevated in the cases (6.72 ± 1.05%) compared to the controls (5.14 ± 0.28%), reflecting poor glycemic status among the GDM group (p < 0.001). Additionally, mean vitamin D levels were significantly lower among cases (10.15 ± 5.85 ng/mL) compared to controls (19.22 ± 7.34 ng/mL), indicating pronounced vitamin D deficiency associated with GDM (p < 0.001). Correspondingly, the prevalence of vitamin D deficiency was substantially higher in the GDM group (90.0%) than in the control group (60.0%), further reinforcing the inverse relationship between vitamin D status and GDM (p < 0.001). These findings strongly suggest that reduced vitamin D levels are associated with

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impaired glucose metabolism and increased risk of gestational diabetes mellitus.

Figure 1: Comparison of Maternal age between Cases and controls

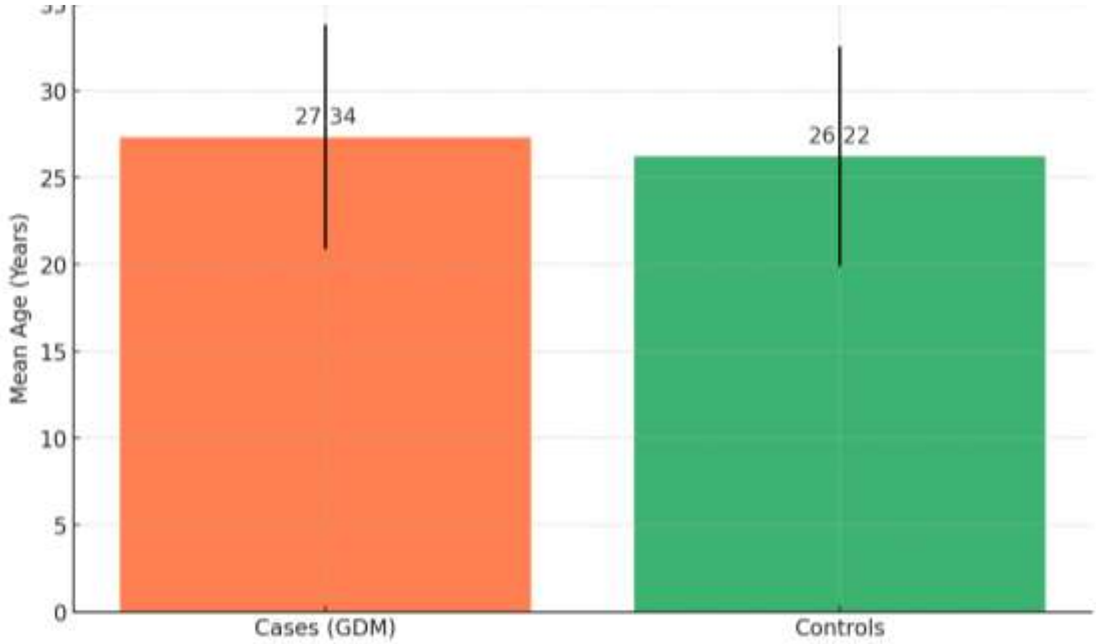

Figure 1 illustrates the comparison of maternal age between the GDM cases and the control group. The mean maternal age in the case group was slightly higher (27.34 ± 6.44 years) compared to the control group (26.22 ± 6.34 years); however, this difference was not statistically significant ($p > 0.05$). This suggests that maternal age, within the studied range, did not have a significant association with the development of gestational diabetes in this population.

Figure 2: Serum vitamin D level in cases and controls

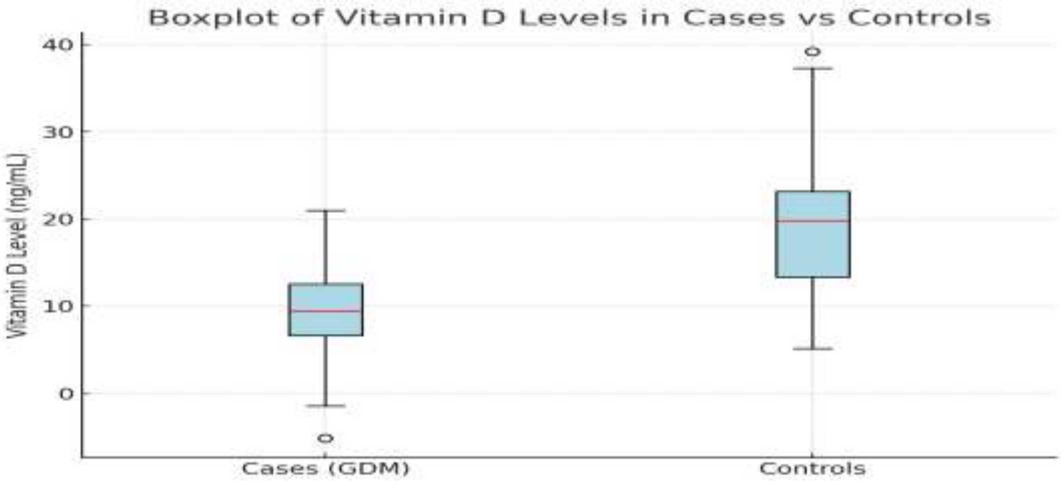

Figure 2 presents a boxplot comparison of serum Vitamin D levels between women with gestational diabetes mellitus (GDM) (Cases) and those with normal glucose tolerance (Controls). The median Vitamin D level in the GDM group is notably lower than that in the control group, indicating a significant

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difference in Vitamin D status. The interquartile range (IQR) in the case group is also narrower and shifted toward the deficient range, while the control group displays a broader range with higher median and upper quartile values. Several outliers with very low Vitamin D levels are visible in the GDM group, further suggesting severe deficiency in some individuals. In contrast, the control group shows fewer outliers and generally higher Vitamin D values. This boxplot demonstrates a significant inverse association between Vitamin D levels and the presence of GDM, supporting the hypothesis that Vitamin D deficiency may be a contributing factor in the pathogenesis of gestational diabetes.

Fig 3: Scatter plot of Vitamin D level vs HBA1C in cases and controls

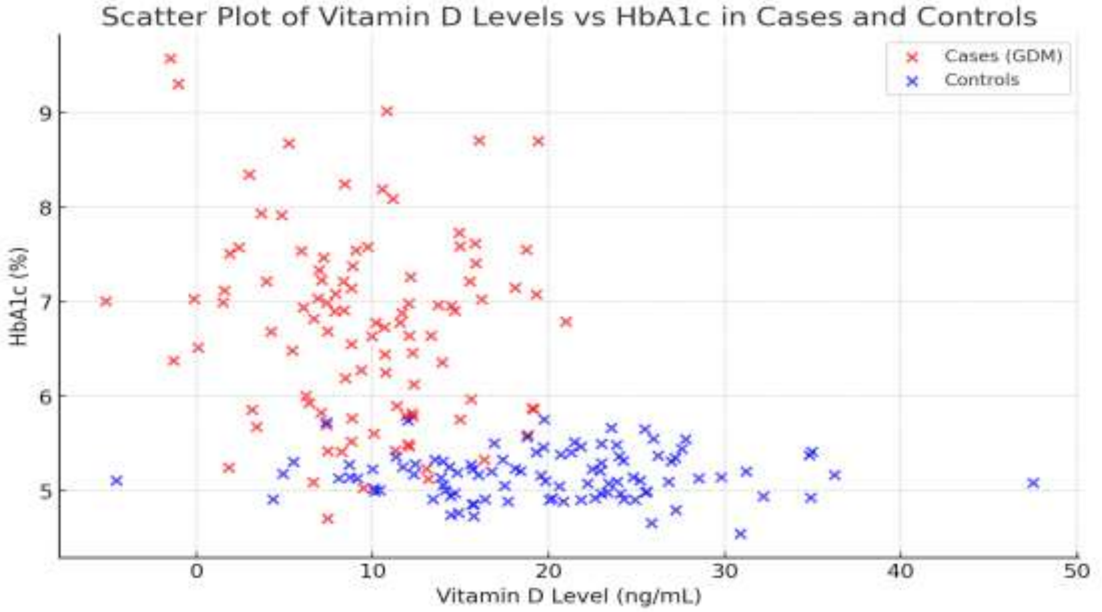

Figure 3 displays a scatter plot illustrating the relationship between Vitamin D levels (ng/mL) and HbA1c (%) among pregnant women with GDM (Cases, red dots) and those with normal glucose tolerance (Controls, blue dots). In the GDM group, an inverse relationship is evident—lower Vitamin D levels are associated with higher HbA1c values, indicating poorer glycemic control. The red cluster is concentrated in the lower Vitamin D and higher HbA1c range, reinforcing the link between Vitamin D deficiency and elevated blood glucose levels in GDM patients. In contrast, the control group shows tighter clustering of data points at higher Vitamin D levels and lower HbA1c percentages, suggesting better glycemic control. The distribution of the control group appears more consistent, with fewer extreme values and less variation in HbA1c.

Discussion

The current study's findings on the association between serum vitamin D levels and gestational diabetes mellitus (GDM) align with several recent studies, reinforcing the potential link between vitamin D deficiency and GDM.

In the present study, the mean maternal age was similar between cases (27.34 ± 6.44 years) and controls (26.22 ± 6.34 years; $p > 0.05$). Dietary habits were comparable, with vegetarian diets reported in 11.5% of cases and 9.5% of controls, and non-vegetarian diets in 88.5% and 90.5%, respectively ($p > 0.05$). A significant difference was found in the history of GDM, higher in cases (9.0%) than controls (1.0%; $p < 0.01$). Family history of type 2 diabetes showed no significant difference (cases: 9.5%, controls: 7.0%; $p > 0.05$). In a cross-sectional study by Anupriya Narain et al. (2022)[10], it was similarly noted that although maternal age was higher among GDM patients (mean age 27.8 years), the difference was not statistically significant compared to controls. Furthermore, Iqbal Suhail et al. (2023)[11] investigated the link between serum vitamin D levels and GDM and also found no significant differences in dietary patterns or family history of diabetes between cases and controls, suggesting that these variables may not be independently associated with GDM risk. However, their study emphasized that a prior history of GDM was more common in vitamin D-deficient

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women, indicating that both vitamin D status and obstetric history may interact as cumulative risk factors. Additionally, Jayaraman Muthukrishnan and Dhruv Goel (2021)[12,] in a study evaluating maternal risk factors for GDM about vitamin D levels, also found that past history of GDM was significantly more prevalent among women with both GDM and low serum vitamin D, suggesting a possible compounding effect of these factors.

These comparisons with current literature strengthen the interpretation that while maternal age, diet, and family history may not differ significantly between groups, a prior history of GDM is a consistent and significant risk factor, particularly in the context of low vitamin D status, further supporting the biological plausibility of vitamin D deficiency as a contributing factor to GDM pathophysiology.
The present study shows that Adequate calcium intake was slightly lower in cases (63.0%) than controls (71.0%), and periconceptional multivitamin use was 26.0% vs. 31.0%, respectively—both differences were not statistically significant ($p > 0.05$). However, sunlight exposure (>3 hours/day) was significantly lower among cases (8.0%) compared to controls (32.0%; $p < 0.01$), suggesting limited sun exposure may be linked to lower vitamin D levels in GDM cases. This finding is consistent with the study by Jayaraman Muthukrishnan and Goel Dhruv,[13,] which highlighted the role of sunlight exposure in maintaining adequate vitamin D levels during pregnancy.

The present study reveals significantly higher fasting blood sugar (95.34 ± 6.98 vs. 79.42 ± 4.67 mg/dL) and HbA1c levels (6.72 ± 1.05% vs. 5.14 ± 0.28%) in GDM cases compared to controls ($p < 0.001$). Vitamin D levels were markedly lower in cases (10.15 ± 5.85 ng/mL) than in controls (19.22 ± 7.34 ng/mL), with a higher prevalence of deficiency in the GDM group (90.0% vs. 60.0%; $p < 0.001$). These findings indicate a strong inverse association between vitamin D status and GDM risk. These findings align with the study by Anupriya Narain et al.,[10,] which reported lower serum vitamin D levels in GDM patients compared to non-GDM pregnant women. Furthermore, the study by Suhail Iqbal et al.[11] supports these observations, demonstrating a significant association between low maternal serum vitamin D levels and the development of GDM. Their research indicated that vitamin D deficiency could be a contributing factor to impaired glucose metabolism during pregnancy.

In the present study, the median Vitamin D level is significantly lower in the GDM group, with a narrower IQR shifted toward deficiency. Outliers with very low levels are more frequent among cases, while controls show a broader range and higher values. This highlights a strong inverse association between Vitamin D levels and GDM, supporting its potential role in GDM pathogenesis. In the present study, the median Vitamin D level was significantly lower in women with gestational diabetes mellitus (GDM), with a narrower interquartile range (IQR) and more frequent outliers indicating severe deficiency. This finding aligns with recent evidence suggesting a strong inverse association between serum Vitamin D levels and GDM. In GDM cases (red dots), lower Vitamin D is associated with higher HbA1c, indicating poor glycemic control. These points cluster in the low Vitamin D–high HbA1c region. In contrast, controls (blue dots) cluster at higher Vitamin D and lower HbA1c levels, showing better glycemic stability with fewer outliers. This shows that Lower serum vitamin D levels in GDM patients corresponded with higher HbA1c and fasting glucose levels, suggesting a possible role of vitamin D in modulating insulin sensitivity and glucose metabolism. For instance, Zhu et al. (2023)[14,] in a multicenter prospective cohort study published in Nutrients, reported that pregnant women with low 25(OH)D levels (<20 ng/mL) in the first trimester had a significantly higher risk of developing GDM, with the lowest quartile associated with a 1.65-fold increased risk compared to the highest quartile. Similarly, Wang et al. (2022)[15] observed in their case-control study that mean serum Vitamin D levels were markedly lower in GDM patients compared to healthy pregnant controls, and this deficiency was independently associated with impaired glucose regulation (Journal of Endocrinological Investigation). Additionally, Zhao H et al. (2023)[16] demonstrated in their Chinese cohort that vitamin D deficiency (<20 ng/mL) was significantly more prevalent among GDM cases, and was correlated with elevated HbA1c levels and reduced insulin sensitivity. Together, these studies support the findings of the present research and reinforce the hypothesis that Vitamin D deficiency may play a contributory role in the pathogenesis of GDM through mechanisms involving altered insulin secretion and resistance.

Generalizability

The findings are mainly applicable to pregnant women in tertiary care settings similar to South Bihar. As a single-center study, broader applicability is limited, with unaccounted factors like ethnicity, climate, and healthcare access potentially influencing

results. However, alignment with other studies supports their relevance, warranting larger multicenter research to improve generalisability.
Limitations: This single-center study has limited generalisability, and while the sample size was adequate, it restricted subgroup analysis. Its case-control design prevents causal inference. Seasonal variation, BMI, and socioeconomic factors were not fully accounted for.

Conclusion

This study highlights a significant association between low serum vitamin D levels and the presence of gestational diabetes mellitus (GDM). While demographic and dietary factors such as maternal age, diet type, and family history of diabetes showed no significant differences between groups, a past history of GDM and reduced sunlight exposure were more common among GDM cases. Biochemically, GDM patients exhibited markedly lower vitamin D levels and poorer glycemic control (higher fasting blood sugar and HbA1c) compared to controls. These findings support the potential role of vitamin D deficiency in the pathogenesis of GDM and underscore the need for further research to evaluate vitamin D supplementation as a preventive strategy during pregnancy.

Acknowledgement

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List of abbreviations

GDM – Gestational Diabetes Mellitus
25(OH)D – 25-Hydroxyvitamin D
HbA1c – Glycated Hemoglobin
BMI – Body Mass Index
IQR – Interquartile Range
CI – Confidence Interval
OR – Odds Ratio
UV – Ultraviolet

Funding

The study received no funding.

Conflict of interest

The authors declare no conflict of interest.

Author biography

Dr. Poojita is an Associate Professor in the Department of Obstetrics and Gynaecology at Narayan Medical College and Hospital, Jamuhar, with expertise in antenatal care and gestational diabetes.

Dr. Renuka Keshari is a Professor in the same department, with extensive experience in obstetrics, endocrinology in pregnancy, and women's health research.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request. All data were collected and managed by institutional ethical guidelines, ensuring participant confidentiality.

Authors' contributions

Dr. Poojita was responsible for the study conception, design, data collection, and statistical analysis. She also contributed to the interpretation of results and drafting of the manuscript.

Dr. Renuka Keshari provided critical guidance in study design, supervised the research process, and contributed to the interpretation of findings and manuscript revision. Both authors read and approved the final manuscript.

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