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Correlation between serum vitamin D levels, glycemic variability, and insulin resistance in prediabetic subjects. A retrospective study.

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Abstract
Background
Impaired glucose tolerance or impaired fasting glucose are hallmarks of prediabetes, a crucial transitional stage that frequently precedes Type 2 Diabetes Mellitus (T2DM). There is growing evidence that glucose homeostasis is significantly impacted by deficiencies in micronutrients, especially vitamin D (25-hydroxyvitamin D). In India, vitamin D insufficiency is still common despite the country's abundant sunshine.
Objective
In a prediabetic cohort in Keonjhar, Odisha, this study attempts to assess the relationship between serum vitamin D levels, insulin resistance (HOMA-IR), and glycemic variability.
Methods
Over the course of a year, a retrospective observational study was carried out at a tertiary medical facility in Keonjhar. 250 participants with prediabetes (HbA1c 5.7–6.4%) between the ages of 30 and 60 were enrolled in the study. Medical records were consulted for information on anthropometry, fasting plasma glucose (FPG), post-prandial glucose (PPG), HbA1c, fasting insulin, and serum 25(OH)D. The Homeostatic Model Assessment (HOMA-IR) was used to calculate insulin resistance. The standard deviation of FPG (SD-FPG) over the follow-up period was used to measure glycemic variability (GV).
Results
Widespread insufficiency was shown by the mean blood vitamin D level of $18.4 \pm 6.2\, ng/mL$. Vitamin D levels and HOMA-IR showed a significant inverse connection ($r = -0.42, p < 0.001$). Additionally, glycemic variability (SD-FPG) was considerably higher in patients with severe vitamin D insufficiency ($< 10\, ng/mL$) than in those with sufficient levels ($p = 0.001$).
Conclusion
In prediabetic individuals in Keonjhar, vitamin D deficiency is substantially linked to increased insulin resistance and unstable glycemic control. In this area, treating hypovitaminosis D may be a good way to postpone the onset of type 2 diabetes.
Recommendation
Given the significant frequency of vitamin D insufficiency and its correlation with metabolic dysregulation in prediabetes, including vitamin D status assessment into standard prediabetes screening techniques may provide a cost-effective means for early metabolic risk stratification. Additional prospective studies are necessary.

Keywords: *Serum Vitamin D, Glycemic Variability, Insulin Resistance, Prediabetic, Retrospective Study*
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Introduction

One of the biggest public health issues of the twenty-first century is the rising prevalence of Type 2 Diabetes Mellitus (T2DM). India, often referred to as the "diabetes capital of the world," is seeing a fast epidemiological shift in which people with metabolic illnesses are moving from wealthy metropolitan areas to semi-urban and rural areas. A crucial treatment window is represented by prediabetes, an intermediate condition of hyperglycemia with glycemic indices above normal but below the diabetes threshold [1].

The distinct "Asian Indian Phenotype," which is defined by greater visceral adiposity and insulin resistance at lower Body Mass Indices compared to Western populations, is responsible for the startlingly high conversion rate from prediabetes to overt diabetes [2].

Prediabetes is caused by two primary pathophysiological defects: a progressive loss of β-cell function in the pancreas and insulin resistance in peripheral organs, including skeletal muscle, liver, and adipose tissue. The significance of micronutrients has attracted a lot of scientific attention, even though lifestyle, sedentary behavior, and genetic predisposition are the main causes. In particular, vitamin D (25-hydroxyvitamin D) is now understood to be a powerful steroid hormone with pleiotropic extraskeletal effects rather than only a regulator of calcium and bone metabolism.

The Role of Vitamin D in Glucose Homeostasis

The identification of Vitamin D Receptors (VDR) and the enzyme 1α-hydroxylase within pancreatic β-cells supports the biological plausibility of Vitamin D regulating glucose metabolism. This implies that insulin secretion is directly influenced by vitamin D in an autocrine or paracrine manner [3]. By increasing the expression of insulin receptors and boosting glucose transport (GLUT-4) activity in muscle and adipose tissue, vitamin D is thought to mechanistically increase insulin sensitivity. Moreover, vitamin D controls the flow of calcium across cell membranes. Chronic vitamin D deficiency (VDD) causes secondary hyperparathyroidism and changed intracellular calcium levels, which might impede insulin signal transduction and attenuate the initial phase of insulin release because insulin secretion is a calcium-dependent process [4, 5].

The Indian Paradox and Regional Context

India has a bizarre epidemic of vitamin D deficiency, with prevalence rates varying from 70% to 90% in different population subsets, despite being a tropical country with plenty of sunshine. This condition is caused by a number of causes, such as darker skin color (where higher melanin lowers UVB absorption), conservative clothing patterns, and growing urbanization that restricts outdoor activities. Furthermore, the Indian cuisine is frequently high in phytates, which might prevent the body from absorbing calcium and vitamin D [6].

Odisha's mineral-rich region of Keonjhar has a distinct environmental and demographic profile. Tribal communities, miners, and urban residents make up the diverse population. Hypovitaminosis may be caused by occupational dust, pollution, and avoiding the intense noon light, even though one may believe that sun exposure is higher in such an area than in larger cities. There is currently a dearth of information precisely analyzing the relationship between metabolic indicators and vitamin D levels in the prediabetic population in this particular area.

Glycemic Variability: An Emerging Risk Factor

Glycemic variability (GV), or the amplitude and frequency of glucose variations, is not captured by HbA1c, which is still the gold standard for evaluating chronic glycemic control because it is an average of the preceding three months. High GV may cause oxidative stress and endothelial dysfunction more severely than persistent chronic hyperglycemia, according to recent research [7]. Vascular damage and "metabolic memory" can be brought on by abrupt changes in blood glucose levels. Examining whether vitamin D levels affect these variations may open up new options for controlling prediabetes and averting microvascular problems.

Objectives

The following goals guided the conduct of this retrospective study:

- To determine the prevalence of vitamin D insufficiency in a Keonjhar cohort of people with prediabetes.

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- To examine the relationship between insulin resistance (HOMA-IR) and serum vitamin D levels.
- To evaluate the connection between glycemic variability (as measured by SD-FPG) and vitamin D status.

Materials and Methods

Study Design, Setting, and Population

The Department of General Medicine at a tertiary care hospital in Keonjhar, Odisha, served as the foundation for this retrospective observational study. The institution manages a varied patient load that represents the district's socioeconomic range while acting as a key referral center.

Study Setting

A thorough examination of medical records produced throughout a 12-month period that is, from January 1, 2023, to December 31, 2023 was included in the study period. Between January and March of 2024, the real data extraction and analysis were completed.

To guarantee the validity of our cohort, we put in place a thorough screening procedure. Based on the anticipated 80% prevalence of vitamin D deficiency among patients with metabolic syndrome in Eastern India, the target sample size was established.

Participants

A sample size of 250 eligible patients using normal statistical power estimates with a 5% precision margin and accounting for possibly partial information typical of retrospective research.

Subject Selection and Criteria

In order to find people between the ages of 30 and 60 who had been diagnosed with prediabetes in accordance with the American Diabetes Association (ADA) criteria, a thorough examination of patient files was required [8]. Patients having a Fasting Plasma Glucose (FPG) between 100 and 125 mg/dL or a HbA1c between 5.7% and 6.4% were included in this. We only included patients who had lived in the Keonjhar district for at least three years in order to make sure the study reflected the local environmental context.

To exclude confounding factors that can distort the metabolic or hormonal profile, we used strict exclusion criteria. Atypical antipsychotics and systemic corticosteroids, which are known to change glucose metabolism, were excluded, as were people with established Type 1 or Type 2 Diabetes Mellitus. Additionally, in order to prevent artificially elevated serum levels, we screened for and excluded patients who had used calcium or vitamin D supplements during the three months before to testing. Additionally, because these disorders significantly change the hydroxylation and metabolism of vitamin D, patients with chronic liver illness or chronic kidney disease ($eGFR < 60\ mL/min$) were excluded. Finally, because of the physiological changes in insulin sensitivity linked to these conditions, women who were pregnant or nursing were not included.

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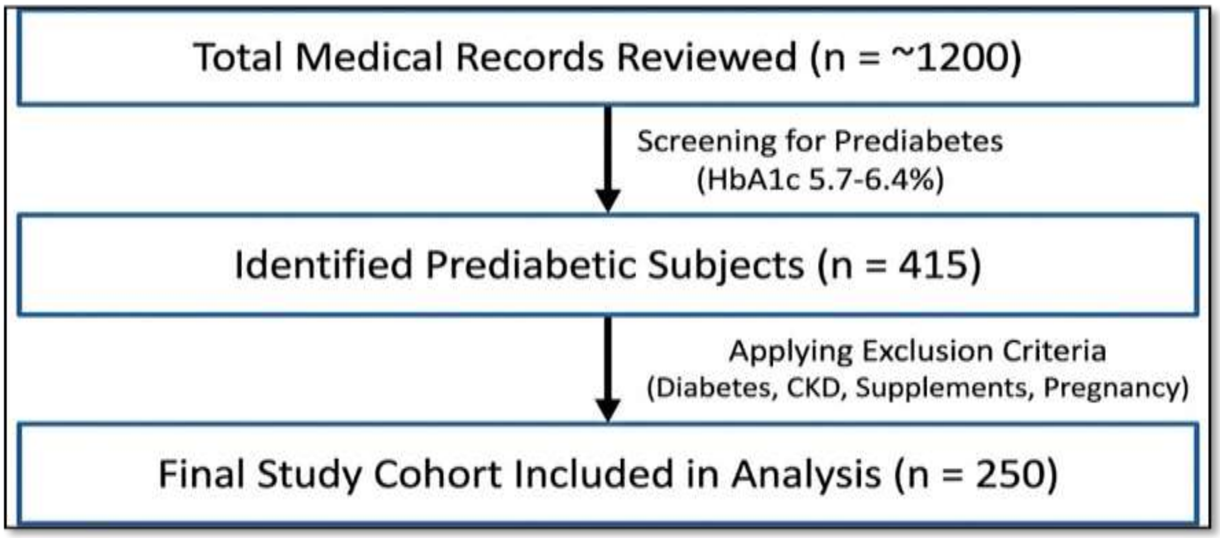

Figure 1 Flowchart illustrating the subject selection and exclusion process.

Data Acquisition and Laboratory Analysis

The Hospital Information System (HIS) was the source of all pertinent clinical data. Age, gender, and place of residence (rural versus urban) were among the demographic information we compiled. Anthropometric measurements were taken, including height, weight, and Body Mass Index (BMI), which was categorized in accordance with Asian Indian-specific criteria, where a BMI greater than 23 kg/m^2 denotes overweight status.

During the study period, the hospital used normal laboratory procedures for biochemical analysis. The hexokinase technique, which provides high specificity, was used to quantify fasting plasma glucose (FPG) and 2-hour post-prandial glucose (PPG). High-Performance Liquid Chromatography (HPLC), the gold standard for long-term glucose monitoring, was used to test glycated hemoglobin (HbA1c). The Chemiluminescence Immunoassay (CLIA) method, which is renowned for its sensitivity in hormonal assays, was used to measure serum 25-hydroxyvitamin D [25(OH)D] and fasting insulin levels. For analysis, a typical lipid profile comprising total cholesterol, triglycerides, HDL, and LDL was also taken out.

Definitions and Statistical Methodology

The Endocrine Society criteria [9] were used to classify vitamin D status for this investigation. Serum levels below 20 ng/mL were considered deficient, levels between 20 and 29 ng/mL were considered insufficient, and levels equal to or more than 30 ng/mL were considered sufficient. The Homeostatic Model Assessment (HOMA-IR), which is computed as Fasting Insulin ($\mu U/mL$) multiplied by Fasting Glucose (mg/dL) divided by 405, was used to quantify insulin resistance mathematically [10].

In retrospective investigations without Continuous Glucose Monitoring (CGM), Glycemic Variability (GV) poses a problem. We used the Standard Deviation of Fasting Plasma Glucose (SD-FPG) to estimate GV in order to address issue. This measure, which serves as a stand-in for longitudinal glucose variation, was only computed for patients who had at least three different FPG readings taken over the course of the one-year study period.

Statistical analysis

SPSS Version 25.0 was used to process the data. While categorical variables were represented as percentages, continuous variables were reported as Mean $\pm$ Standard

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Deviation (SD). To investigate the linear correlations between Vitamin D, HOMA-IR, and GV, we used Pearson's correlation coefficient and the independent t-test and ANOVA for group comparisons. To account for possible confounders including age and BMI, multivariate linear regression was used; a p-value of less than 0.05 was deemed statistically significant.

3. Results

3.1 Demographic and Baseline Characteristics

The mean age of the 250 individuals in the final analysis was 45.2 ± 8.5 years. Males made up 58% of the sample, indicating a male predominance in the cohort. Given the metabolic makeup of the prediabetic group, the mean BMI for Asian Indians was determined to be $26.4 \pm 3.1\ kg/m^2$. This falls into the overweight/obese category. With a mean fasting glucose of $112.5 \pm 9.4\ mg/dL$ and a mean HbA1c of $6.0 \pm 0.3\%$, the baseline biochemical values showed typical prediabetic profiles.

Table 1 Baseline Characteristics of the Study Population (n=250)

Parameter	**Mean ± SD**	**Reference Range**
Age (years)	45.2 ± 8.5	–
BMI (kg/m^2)	26.4 ± 3.1	$18.5 - 22.9$\$(Asian)$
Fasting Glucose (mg/dL)	112.5 ± 9.4	< 100
HbA1c (%)	6.0 ± 0.3	< 5.7
Fasting Insulin ($\mu U/mL$)	14.2 ± 5.1	$2.6 - 24.9$
Serum 25(OH)D (ng/mL)	18.4 ± 6.2	> 30
HOMA-IR	3.9 ± 1.4	< 2.5

Vitamin D Status and Group Comparisons

A widespread problem with vitamin D levels in the sample population was identified by the investigation. 28% (n=70) were deemed insufficient, while a startling 62% (n=155) were deemed deficient ($< 20\ ng/mL$). Merely 10% (n = 25) attained adequate levels ($\geq 30\ ng/mL$). The average serum vitamin D level was $18.4 \pm 6.2\ ng/mL$. The mean levels of vitamin D were significantly lower in female individuals ($16.1 \pm 5.4\ ng/mL$) than in male subjects ($19.8 \pm 6.5\ ng/mL$).

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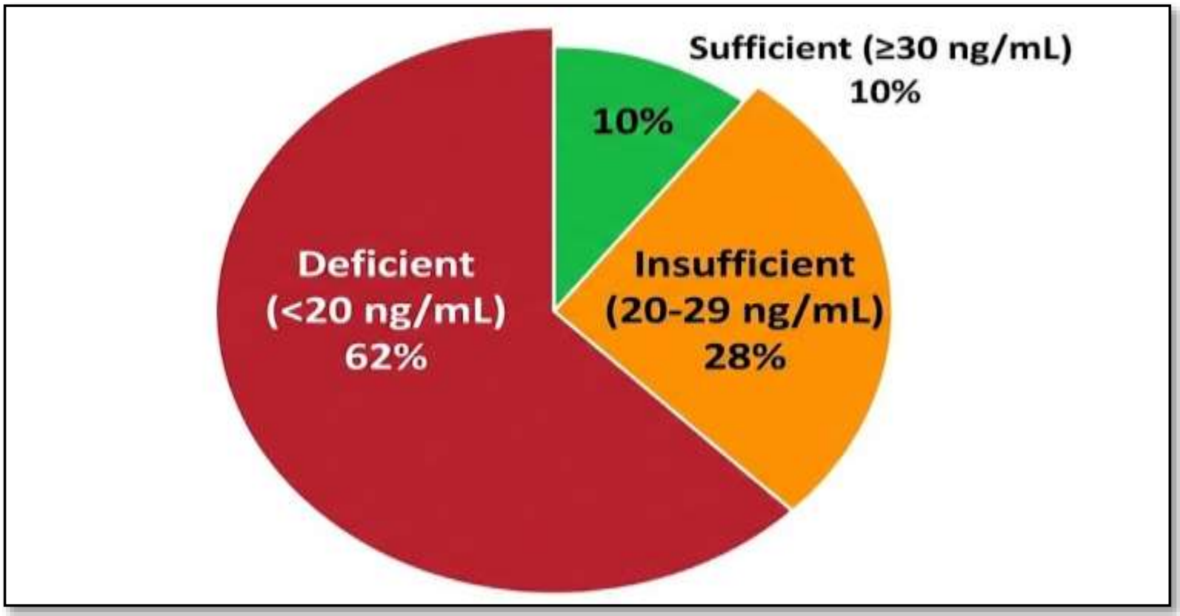

Figure 2 Prevalence of Vitamin D deficiency, insufficiency, and sufficiency

We divided the patients into two groups in order to better understand the metabolic effects of vitamin D: Group A (Deficient, $< 20\ ng/mL$) and Group B (Non-Deficient, $\geq 20\ ng/mL$). Patients with vitamin D insufficiency exhibited significantly higher fasting insulin levels and HOMA-IR scores, indicating poorer insulin resistance, as seen in Table 2. Furthermore, the deficient group's lipid profile revealed a tendency toward dyslipidemia, with noticeably elevated triglyceride levels.

Table 2 Comparison of Metabolic Parameters between Vitamin D Deficient and Non-Deficient Groups

Parameter	**Group A: Deficient ($< 20\ ng/mL$) (n=155)**	**Group B: Non-Deficient ($\geq 20\ ng/mL$) (n=95)**	**p-value**
Fasting Insulin ($\mu U/mL$)	16.8 ± 4.5	10.1 ± 3.2	$< \mathbf{0.001}$
HOMA-IR	4.6 ± 1.3	2.8 ± 0.9	$< \mathbf{0.001}$
Total Cholesterol (mg/dL)	198.5 ± 32.1	182.4 ± 28.5	0.045
Triglycerides (mg/dL)	175.2 ± 45.6	148.3 ± 38.2	$\mathbf{0.012}$
HDL Cholesterol (mg/dL)	38.5 ± 5.2	44.1 ± 6.1	0.038

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Correlation with Insulin Resistance and Glycemic Variability

Serum 25(OH)D levels and HOMA-IR showed a statistically significant negative connection ($r = -0.42, p < 0.001$). This implies that insulin resistance gradually gets worse as vitamin D levels drop. The mean HOMA-IR of subjects in the lowest quartile of vitamin D was nearly double that of those in the highest quartile.

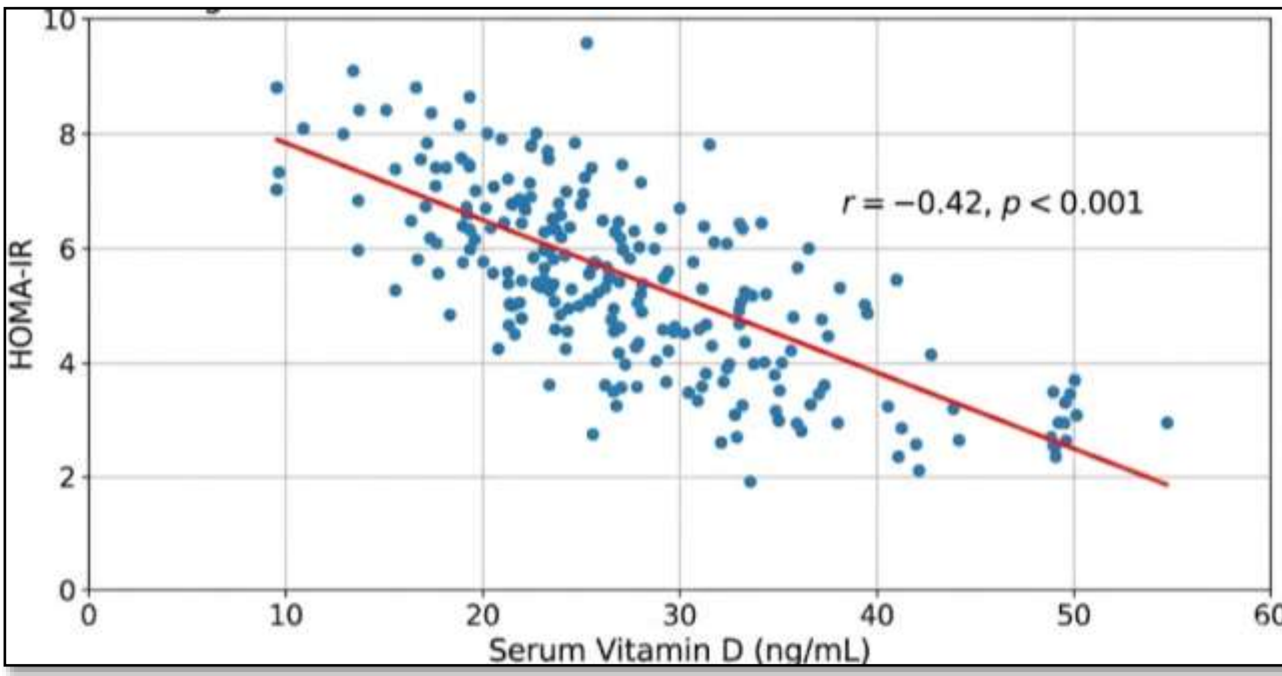

Figure 3 Scatter plot demonstrating the inverse correlation between Serum Vitamin D and HOMA-IR

Vitamin D insufficiency is linked to unstable glucose regulation, according to data on Glycemic Variability, which was measured by the SD-FPG throughout the course of the year. The Glycemic Variability is broken down according to the degree of vitamin D insufficiency in Table 3, which displays a distinct gradient where lower vitamin D levels are associated with higher fasting glucose standard deviations.

Table 3 Glycemic Variability (SD-FPG) across Vitamin D Status Categories

Vitamin D Status	Mean Serum Vit D (ng/mL)	Mean SD-FPG (mg/dL)	95% CI for SD-FPG
Severe Deficiency ($< 10\ ng/mL$)	8.2 ± 1.5	19.4 ± 4.8	$17.1 - 21.7$
Deficiency ($10 - 19\ ng/mL$)	15.6 ± 2.8	16.1 ± 3.9	$14.8 - 17.4$
Insufficiency ($20 - 29\ ng/mL$)	24.1 ± 2.5	12.3 ± 3.0	$11.2 - 13.4$

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Sufficiency ($\geq 30\ ng/mL$)	34.5 ± 4.1	9.8 ± 2.1	$8.9 - 10.7$

Discussion

The current retrospective study highlights the widespread prevalence of vitamin D deficiency (VDD) among prediabetic people and provides a critical analysis of the metabolic landscape in Keonjhar, Odisha. Our findings demonstrate a strong correlation between increased glycemic instability, worsening insulin resistance, and hypovitaminosis D.

The "Indian Paradox" of widespread deficiency in spite of plenty of sunlight is consistent with the startling result that 90% of the research population had either insufficient or deficient levels of vitamin D. The prevalence estimates are consistent with those found in North India by Marwaha et al. [11]. One may anticipate more sun exposure in Keonjhar, a semi-urban area with a reputation for mining operations, than in urban areas. However, the advantages of the region's latitude are probably offset by environmental factors including suspended particulate matter from mining, which scatters UVB rays, and cultural practices of seeking shade and covering the body to avoid the extreme tropical heat. Additionally, the local food provides little in the way of dietary Vitamin D compensation because it is primarily rice-based and contains nothing in the form of fortified dairy products or fatty fish [12].

The mechanistic ideas put forth by researchers such as Dutta et al. [13] and more recently by Park et al. [14] are supported by the inverse association ($r = -0.42$) between Vitamin D and HOMA-IR found in this study. The pancreatic oversecretes insulin in the prediabetic state in an effort to make up for peripheral insulin resistance. Because it controls the intracellular calcium flux in β-cells, which initiates insulin exocytosis, vitamin D is crucial for this process. Therefore, a shortage has two effects: it may lower peripheral tissue expression of insulin receptors while also impairing the pancreas' capacity to mount a successful secretory response. Additionally, Vitamin D possesses potent anti-inflammatory properties. Since insulin resistance is fueled by systemic low-grade inflammation and cytokine storms (involving TNF-α and IL-6), Vitamin D deficiency may leave this inflammatory state unchecked, further degrading insulin signaling pathways.

The relationship between vitamin D insufficiency and Glycemic Variability (GV), as measured here by SD-FPG, may be this study's most innovative contribution. Although HbA1c is a good indicator of average blood sugar, it obscures the daily highs and lows that define "brittle" metabolic regulation. The discovered that SD-FPG was considerably better in VDD participants, suggesting that their fasting glucose fluctuates more dramatically. This is consistent with research by Alvarez et al. [5], who proposed that vitamin D administration helped regulate fasting glucose levels. Because fast glucose fluctuations more aggressively produce oxidative stress and endothelial damage than stable hyperglycemia, this stability is clinically crucial. Vitamin D-deficient prediabetics may be more susceptible to early neuropathy or retinopathy even prior to a formal diagnosis of diabetes, as oxidative stress is a major cause of microvascular problems [15].

From a clinical standpoint, these results are very important for locations like Keonjhar that have little resources. The significant incidence of vitamin D deficiency indicates that empirical supplementation may be a cost-effective public health strategy, even though mass screening for the vitamin may be unaffordable [16]. As a type of "nutritional modulation," adding calcium and vitamin D supplements to the normal treatment regimen for prediabetes may flatten the HOMA-IR curve and stabilize glucose levels without requiring strong pharmaceutical intervention.

Limitations

Even though this study provides insightful information, it is crucial to consider its limitations. First and foremost, the retrospective design makes it possible to find high correlations but prevents us from establishing clear causation; without a prospective interventional trial, we cannot demonstrate that correcting the deficiency will restore the insulin resistance. Second, the Standard Deviation of Fasting Plasma Glucose (SD-FPG) obtained from sporadic hospital visits was the basis for our evaluation

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of Glycemic Variability. Although this is a valid substitute, it is not as precise as Mean Amplitude of Glycemic Excursions (MAGE) or Continuous Glucose Monitoring (CGM), which would detect postprandial spikes that FPG ignores.

Furthermore, because this is a single-center study, there is an inherent selection bias; patients who visit a tertiary care facility might be more health-conscious or have more comorbidities than the overall rural population of Keonjhar. Lastly, even though we statistically controlled for BMI, we were unable to retrospectively account for lifestyle factors that could potentially confound vitamin D metabolism, such as precise levels of physical activity, seasonal variations in sun exposure at the precise time of blood sampling, or specific dietary calcium intake.

Conclusion

In conclusion, this study offers region-specific data from Keonjhar, Odisha, demonstrating that vitamin D deficiency is widespread in prediabetic people and is not just an incidental finding. The results show a strong inverse relationship between blood vitamin D levels and insulin resistance (HOMA-IR), as well as a strong correlation with increased glycemic variability. These findings suggest that vitamin D insufficiency is a "silent collaborator" in the development of metabolic syndrome.

In the treatment of prediabetes, these results imply that vitamin D status should be considered a modifiable risk factor. When patients in this area present with decreased glucose tolerance, clinicians should be highly suspicious of VDD. The remediation of this insufficiency by dietary changes, targeted sunshine exposure.

Recommendation

Considering the high prevalence of vitamin D deficiency and its association with metabolic dysregulation in prediabetes, incorporating vitamin D status evaluation into conventional prediabetes screening methods may offer a cost-effective approach for early metabolic risk assessment. Further prospective research is required.

Conflict of interest

The authors have no conflict of interest.

List of abbreviations

Type 2 Diabetes Mellitus	T2DM
Vitamin D Receptors	VDR
Glucose transport	GLUT-4
Vitamin D deficiency	VDD
Glycemic variability	GV
Fasting plasma glucose	FPG
Post-prandial glucose	PPG
American Diabetes Association	ADA
Hospital Information System	HIS
Homeostatic Model Assessment of Insulin Resistance	HOMA-IR
Standard Deviation (SD) of Fasting Plasma Glucose (FPG)	SD-FPG
Chemiluminescence Immunoassay	CLIA
Continuous Glucose Monitoring	CGM
Analysis of variance	ANOVA
Standard Deviation	SD

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