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Vitamin D and Calcium in Type-2 Diabetes Mellitus: An Analytic Cross-Sectional Study

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ABSTRACT

Type-2 diabetes mellitus (T2DM) is a complex metabolic disorder, with unclear roles of micronutrients like vitamin D and calcium in its pathogenesis. To evaluate and compare vitamin D and calcium levels in serum of T2DM cases and healthy controls and investigate their associations with plasma glucose levels in T2DM by analyzing correlations. This hospital-based, observational, analytic cross-sectional study included 122 T2DM patients and 122 age-and sex-matched controls. FPG, PPPG, serum vitamin D and calcium levels were measured. Statistical analyses were performed using SPSS® version 28.0.0, with Pearson's correlation and unpaired t-tests. A p-value<0.05 was considered statistically significant and p<0.001 highly significant. FPG (170±52.91 mg/dl vs. 75.88±15.8 mg/dl) and PPPG (216±39.19 mg/dl vs. 111±14 mg/dl) were significantly higher in T2DM (p=0.0001). Vitamin D levels were significantly lower in T2DM (24.23±10.60 ng/ml vs. 28.5±10.23 ng/ml, p=0.038), with a negative correlation with FPG (r=-0.375, p=0.0002). Calcium levels were lower but not statistically significant (9.1±1.61 mg/dl vs. 9.59±2.36 mg/dl, p=0.052) and showed a negative correlation with FPG (r=-0.205, p=0.023), which was statistically significant. A positive correlation between calcium and vitamin D (r=0.344, p=0.000) was statistically highly significant. Deficiencies in vitamin D and calcium may contribute to T2DM development and progression. Vitamin D deficiency potentially contribute to lower calcium levels in T2DM patients. Adequate vitamin D and calcium could support glucose homeostasis and their laboratory measurements may play a critical role in prevention and management of T2DM.

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Key Words
Diabetes, glucose, micronutrient, calcium and vitamin D

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INTRODUCTION

Type-2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by insulin resistance (reduced sensitivity of target tissues such as muscle, liver and adipose tissue to insulin) and impaired pancreatic beta-cell function leading to inadequate insulin secretion to compensate for the resistance. This results in persistent hyperglycemia (elevated blood glucose levels)[1]. Approximately 8.7% of the adult population in India suffers from diabetes, placing the country at the epicenter of the global diabetes crisis [2]. Nutritional deficiencies in India, particularly in vitamin D and calcium are widespread both in urban and rural areas[3,4]. Deficiencies of these micronutrients have been recently implicated in the genesis and progression of T2DM implying that vitamin D and calcium might play critical roles in maintaining optimal glucose homeostasis[5]. Deficient vitamin D levels could be associated with several conditions, including heart disease, cancer and more[6]. While some studies indicate a potential interconnection between low vitamin D concentrations and T2DM development[7-9], Other research does not support this connection[10-12]. These conflicting research studies throw a light on the explicit complexity of relationship between T2DM and vitamin D, emphasizing the need for further research to better understand these interactions. Similarly, the essential macromineral calcium may alter the risk of various conditions for eg. cardiovascular disease, stroke, prostate cancer, colorectal cancer, multiple sclerosis, insulin resistance, hyperglycemia, psoriasis, and T2DM[13]. It is noteworthy that studies have shown varying serum calcium levels in T2DM individuals. While some research indicates that T2DM patients have higher serum calcium levels than non-diabetics[14-16], Other investigations have found no association[17,18] and few studies report lower calcium levels in T2DM[19,20]. These mixed and inconsistent results highlight the requirement for further research so as to clarify the relationship between calcium levels and T2DM. Given the growing prevalence of T2DM along with widespread nutritional deficiencies in India, it is therefore imperative to investigate the relationship between the micronutrients and glucose metabolism, especially in light of the contrasting findings reported in various studies. Thus, the present study aims to evaluate and compare the levels of vitamin D and calcium in T2DM subjects and healthy controls and to investigate their associations with glucose levels (fasting plasma glucose ie. FPG and postprandial plasma glucose ie. PPPG) in T2DM by analyzing correlations. By addressing the existing gaps in research pertaining to these micronutrient levels in T2DM, the study seeks to elucidate their potential roles in glucose homeostasis and the genesis of insulin resistance, thereby contributing to a deeper understanding of the complex interrelationship between these micronutrients and T2DM.

MATERIALS AND METHODS

The study was a hospital-based, observational, analytic cross-sectional type conducted in the Department of Biochemistry at Index Medical College, Hospital and Research Centre, Indore after obtaining ethical approval from its Institutional Ethics Committee. The study included 244 participants comprising 122 T2DM patients (cases) and 122 healthy subjects (controls), who were age-and sex-matched and belonged to the age-group of 30-65 years. An informed written consent was obtained from all study participants. T2DM patients were enrolled in the case group based on the diagnostic criteria of the American Diabetes Association[21]. Participants were excluded if they had any acute or chronic illness, pancreatic, hepatic, or renal disease, conditions that could significantly alter blood glucose levels, or if they had used vitamin D or calcium supplements in the past six months. For measuring FPG and PPPG levels, a 2-hour OGTT was performed on 122 cases and 122 controls after a 12-hour overnight fast. Fasting samples (3 ml venous blood) were drawn under aseptic precautions, with 2 ml transferred to a plain vial for vitamin D and calcium estimation and 1 ml to a fluoride vial for FPG analysis. After consuming a 75 gm glucose solution, participants remained seated and refrained from physical activity for 2 hours, after which a 2 ml blood sample was collected in a fluoride vial for PPPG measurement. All vials were inverted 5-10 times to mix the anticoagulants. Blood samples in plain vials were left at room temperature for 30 minutes to clot, while those in fluoride vials were processed immediately. All samples were centrifuged at 2000-3000 rpm for 10-15 minutes to separate serum in plain vials and plasma in fluoride vials[22-24]. The plasma was used to measure FPG and PPPG levels with the ERBA clinical chemistry analyzer by Glucose Oxidase-Peroxidase method[25]. The serum was analyzed for calcium using ERBA clinical chemistry analyzer by O-cresolphthalein complexone method[26,27], while serum vitamin D was estimated using the Beckman Coulter Access-2 immunoassay system by Competitive Chemiluminescent Immunoassay method[28]. For statistical analysis, data were entered into Microsoft® Excel® and analyzed using IBM® SPSS® version 28.0.0. Continuous variables were expressed as mean+standard deviation and their relationships were examined using Pearson's correlation, while differences between two groups were analyzed using Student's unpaired t-test. A p-value of <0.05 was considered statistically significant, while a p-value of <0.001 was deemed highly significant.

RESULTS AND DISCUSSIONS

The present study was conducted with a total of 244 participants, comprising 122 cases of T2DM and 122 healthy controls.

Table 1: Demographic Characteristics in Cases and Controls

Variables		Cases N =122	Controls N =122	(x^2)
Gender	Male	53	51	0.873
	Female	69	71	
Age	30-40	21	34	0.667
	41-50	33	35	
	51-65	68	53	
Area	Rural	71	73	0.765
	Urban	51	49	

The demographic characteristics of cases and controls are illustrated in (Table 1), with chi-square test results for all demographic variables (gender, age and area) showing non-significance (x^2=0.873 for gender, x^2=0.667 for age and x^2=0.765 for area). This indicate that cases and controls are well-matched demographically and are comparable, reducing the likelihood of confounding effects from these variables in further analyses.

Table 2: Tabulated Comparison of FPG, PPPG, Serum Vitamin D and Serum Calcium Between Case and Control Groups

	Type of subjects		
Parameter	**Cases (n=122)**	**Controls (n=122)**	**p-value**
FPG (mg/dl)	170±52.91	75.88±15.8	p= 0.0001**
PPPG (mg/dl)	216±39.19	111±14	p= 0.0001**
Serum vitamin D (ng/ml)	24.23±10.60	28.5±10.23	p=0.038*
Serum calcium (mg/dl)	9.1±1.61	9.59±2.36	p= 0.052 (NS)

*-Statistically significant (p<0.05)
**-Statistically highly significant (p<0.001)
NS-Statistically not significant (p>0.05)

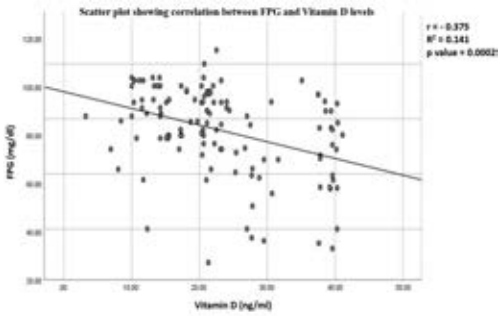
Table 3: Correlation Analysis of FPG, PPPG, Vitamin D and Calcium Levels

	FPG	PPPG	Vitamin D	Calcium
FPG	1	0.054 (0.553)	**- 0.375**** (0.0002)	**- 0.205*** (0.023)
PPPG	-	1	-0.026 (0.778)	0.008 (0.929)
Vitamin D	-	-	1	**0.344**** (0.000)
Calcium	-	-	-	1

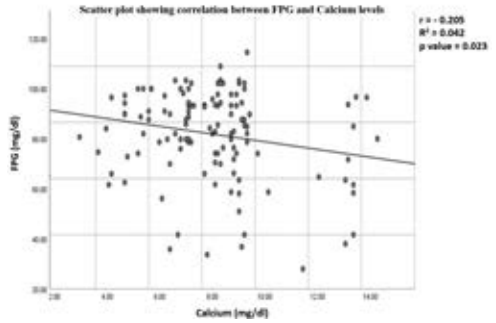
Note:

- **Sample Size:** 122 cases and 122 controls.
- Only the upper triangular part of the table matrix has been shown to remove duplicate values.
- Each cell shows the Pearson's correlation ie. r value followed by p-value in brackets.
- Correlation cells with negligible r value (r≈0) are shaded in light grey to indicate no correlation between the variables.
- *Statistically significant as p<0.05 with r values marked in bold.
- **Statistically highly significant as p<0.001 with r values marked in bold.

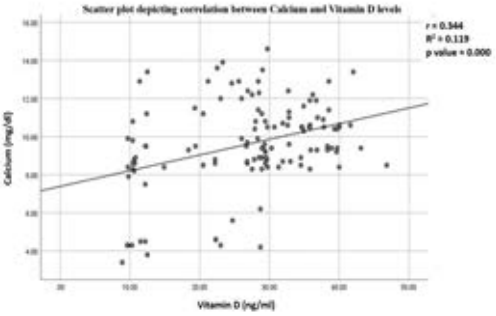
As illustrated in (table-2), FPG (170±52.91 mg/dl vs. 75.88±15.8 mg/dl, p=0.0001) and PPPG (216±39.19 mg/dl vs. 111±14 mg/dl, p=0.0001) levels were significantly higher in cases than controls. Serum vitamin D (24.23±10.60 ng/ml vs. 28.5±10.23 ng/ml, p=0.038) level was significantly low in T2DM. (Table 3) and (Graph-1) depicts a negative correlation between FPG and vitamin D (r=-0.375, p=0.0002), which is statistically highly significant. As shown in table-2, serum calcium (9.1±1.61 mg/dl vs. 9.59±2.36 mg/dl, p=0.052) level was low in T2DM but not statistically significant. Additionally, (Table-3) and (Graph-2) demonstrate a negative correlation between FPG and calcium (r=-0.205, p=0.023), which is statistically significant. Furthermore, the table-3 and (Graph-3) reveal a positive correlation between calcium and vitamin D levels (r=0.344, p=0.000), which is statistically highly significant.



Graph 1: Scatter Plot Showing Correlation Between FPG and Serum Vitamin D Levels



Graph 2: Scatter Plot Demonstrating Correlation Between FPG and Serum Calcium Levels



Graph 3: Scatter Plot Depicting Correlation Between Serum Calcium and Vitamin D Levels

In the present study, the authors have observed statistically significant low vitamin D levels in T2DM patients. This finding is supported by various studies conducted by Zoppini[7], Zhao[8], Kostoglou-

Athanassiou[9], thereby suggesting that reduced vitamin D levels may negatively impact glycemic control. An interventional meta-analysis by Musazadeh V et al. suggested an inverse relationship, as obtained in the present study in the form of a negative correlation between FPG and vitamin D levels[29]. This means that as vitamin D levels decrease in T2DM individuals, FPG levels may increase, thereby highlighting a possible metabolic link between glucose metabolism and vitamin D homeostasis. Moreover, a negative correlation (r=-0.375) suggests that Vitamin D may be one of the contributory factors in regulating glucose metabolism and thereby reduced vitamin D may negatively impact glucose regulation. When vitamin D measurement levels are low, there is less binding to vitamin D receptor (VDR) which leads to impaired vitamin D-VDR signaling that consequently leads to reduced expression of genes involved in insulin synthesis, secretion and receptor expression in pancreatic β-cells. Thus, the downregulation of key genes like IRS1 (insulin receptor substrate 1) and GLUT4 reduces insulin sensitivity in muscle and adipose tissue[30]. Also, in conditions of low vitamin D, β-cells experience increased oxidative stress production, leading to reduced insulin secretion[31]. In the present study, the authors have demonstrated low calcium level in T2DM patients but was statistically non-significant when compared to control groups. Such a finding aligns with the broader understanding that while increased extracellular calcium can enhance calcium influx into β-cells affecting intracellular calcium homeostasis, signaling and insulin secretion[32,33] however, the impact of low extracellular calcium on intracellular dynamics and insulin release remains uncertain. Further research with larger, more diverse subjects is needed to determine whether calcium has a more pronounced effect on glucose homeostasis. Moreover, the observed trend of slightly lower calcium in diabetics, even if not statistically significant, might prompt a routine laboratory measurements of calcium in T2DM patients so as to monitor overall health in T2DM. Numerous studies, including those by Kim[18], Talaei[34] and Tong[35], suggest an inverse relationship between FPG and calcium, which aligns with the negative correlation observed in the present study. This indicates that as calcium levels decline in T2DM individuals, FPG levels may rise, suggesting a potential metabolic link between glucose metabolism and calcium homeostasis, where reduced calcium levels could negatively impact glucose regulation. Additionally, the present study has demonstrated a positive correlation between calcium and vitamin D levels (r=0.344, p=0.000). This indicates that vitamin D has a real effect on calcium levels, thereby suggesting that the lowered levels of serum calcium observed in T2DM patients could be attributed to reduced vitamin D. Low vitamin D induce disruptions in intestinal calcium absorption as vitamin D promotes intestinal absorption of calcium[36]. Furthermore, vitamin D enhances calcium concentrations within pancreatic beta cells, stimulating the translocation of insulin granules to the cell membrane for secretion, thereby improving insulin release[37]. These processes highlight the closely integrated metabolism of vitamin D and calcium and their probable role in the genesis and progression of T2DM.

Limitations: The observational cross-sectional design precludes the establishment of causal relationships, limiting our ability to determine the cause-effect link between micronutrient levels and T2DM. Moreover, the present study lacked dietary data or detailed nutritional records, making it impossible to assess the participants' intake of vitamin D and calcium.

CONCLUSION

The present study observed significantly lower vitamin D in T2DM implicating its potential role in glycemic control, possibly by playing critical roles in the genesis and progression of T2DM. Although calcium levels were lower in T2DM, the differences were not statistically significant, suggesting a subtle trend rather than a definitive relationship, thereby warranting further investigation with larger and more diverse populations. Correlation analyses revealed that both vitamin D and calcium are one of the contributory factors in regulating glucose metabolism, with a significant positive correlation between the two, highlighting the crucial involvement of vitamin D in maintaining calcium homeostasis, which may together influence glycemic control. The authors of the present study hereby suggest that maintaining adequate serum levels and regular assessments of vitamin D and calcium may play a critical role in the prevention and management of T2DM. Attention must be devoted to ensuring sufficient body reserves of these micronutrients, particularly in the context of our country, where deficiencies are widespread. Physicians should be advised to regularly assess the blood levels of these micronutrients while managing glucose control in T2DM patients. Future research, including supplementation trials or longitudinal analyses and especially with larger sample sizes, may lead to advancements in clinical practices and the development of public health recommendations, such

as guidelines for regular laboratory monitoring, dietary intake and supplementation of these micronutrients in T2DM, thereby aiding not only in predicting and preventing the development of T2DM but also in its management.

Conflict of Interest: The authors declare that they have no conflict of interest.

Author Contributions: All authors have equally contributed in this study and have agreed on the final manuscript.

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