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Assessment of serum calcium and phosphate levels in patients with type 2 diabetes mellitus at tertiary care level hospital of Ahmedabad: A cross-sectional study

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Abstract
Background: Type 2 diabetes mellitus (T2DM) is a rapidly growing metabolic disorder worldwide and is associated with multiple biochemical and electrolyte abnormalities. Calcium and phosphate play essential roles in insulin secretion, glucose metabolism, bone health, and cellular signaling. Alterations in these minerals may influence glycemic control and contribute to long-term diabetic complications.
Aim: To evaluate serum calcium and phosphate levels in patients with type 2 diabetes mellitus and compare them with age-matched non-diabetic controls.
Materials and Methods: This cross-sectional study was conducted at a tertiary care center in Ahmedabad and included 30 diagnosed T2DM patients and 30 non-diabetic controls aged 30-80 years. Serum calcium was estimated by the Arsenazo III method, serum phosphate by the Ammonium Molybdate method, and plasma blood glucose by the GOD-POD method using a fully automated analyzer. Data were expressed as mean ± SD and analyzed using the unpaired *t*-test.
Results: Mean serum phosphate (2.2±0.56 mg/dl) and serum calcium levels (8.03±0.3 mg/dl) were significantly lower in T2DM patients compared to controls (3.07±0.21 mg/dl and 8.6±0.3 mg/dl respectively) (p<0.001). Plasma blood glucose levels were significantly higher in diabetic patients.
Conclusion: Hypocalcaemia and hypophosphatemia are significantly associated with hyperglycemia in patients with type 2 diabetes mellitus. Routine monitoring and early correction of these electrolyte imbalances may help improve glycemic control and prevent complications.

Keywords: Type 2 diabetes mellitus, serum calcium, serum phosphate, electrolyte imbalance

Introduction
Diabetes mellitus is one of the most challenging non-communicable diseases affecting global health, with its prevalence rising alarmingly over the past few decades. According to global estimates, the number of individuals with diabetes has more than doubled over the last three decades, making it a major public health concern worldwide. India is projected to be among the countries with the highest burden of diabetes, contributing significantly to diabetes-related morbidity and mortality [1, 2]. The World Health Organization has recognized diabetes mellitus as an emerging epidemic due to its rapid rise and long-term complications [3].
Type 2 diabetes mellitus (T2DM), characterized by insulin resistance and relative insulin deficiency, is associated not only with hyperglycemia but also with multiple metabolic and biochemical derangements. Among these, disturbances in mineral and electrolyte metabolism are increasingly being recognized. Calcium and phosphate are essential minerals involved in glucose metabolism, insulin secretion, bone mineralization, and intracellular signaling [4].
Calcium plays a pivotal role in insulin secretion from pancreatic β-cells, as insulin release is a calcium-dependent process. Altered calcium homeostasis may impair insulin secretion and worsen glycemic control. Chronic hyperglycemia may influence calcium metabolism through increased renal loss, altered vitamin D metabolism, and hormonal dysregulation [5].
Phosphate is a vital component of cellular energy metabolism, participating in Adenosine Triphosphate (ATP) synthesis and glucose phosphorylation. In diabetes mellitus, phosphate depletion may occur due to osmotic diuresis, increased urinary excretion, and reduced intracellular uptake. Hypophosphatemia has been associated with insulin resistance, impaired glucose utilization, and increased risk of diabetic complications [6].
Despite the growing prevalence of diabetes, routine evaluation of mineral disturbances such as hypocalcaemia and hypophosphatemia is often overlooked in clinical practice.

Understanding the relationship between glycemic status and mineral metabolism may provide additional insight into the comprehensive management of patients with T2DM. Hence, the present study was undertaken to assess serum calcium and phosphate levels in patients with type 2 diabetes mellitus and compare them with healthy non-diabetic controls.

Materials and Methods

Study Design and Setting

A hospital-based cross-sectional study was conducted at a tertiary health care center in Ahmedabad over a period of two months from 1st February 2018 to 31st March 2018.

Study Population and Sampling Method

A total of 60 participants were enrolled in the study using a hospital-based convenience sampling method. Thirty diagnosed patients with type 2 diabetes mellitus attending the outpatient and inpatient departments were included as cases. Thirty age- and gender-matched apparently healthy non-diabetic individuals were recruited as controls from among hospital staff attendants and accompanying relatives after confirming normal blood glucose levels.

Inclusion Criteria

For Cases

- Diagnosed patients with type 2 diabetes mellitus, attending the outpatient or inpatient departments of the tertiary care hospital
- Age between 30 and 80 years
- Both male and female participants
- Provided written informed consent

For Controls

- Apparently healthy, non-diabetic individuals matched for age and gender with the cases.
- Age between 30 and 80 years.
- Normal plasma blood glucose levels and no known history of diabetes mellitus.
- Provided written informed consent.

Exclusion Criteria (Applicable to Both Groups)

- History of thyroid disorders (hyperthyroidism or hypothyroidism).
- Chronic renal failure or known renal disease.
- Chronic infections affecting bone, including tuberculosis, osteomyelitis, or chronic bone tumors.
- Hematological disorders and connective tissue disorders.
- Current smokers and chronic alcoholics.
- Participants on medications known to affect calcium or phosphate metabolism (e.g., long-term corticosteroids, Anticonvulsants).

Diagnostic Criteria for Diabetes

Type 2 diabetes mellitus was diagnosed based on any one of the following:-

- Random blood glucose ≥ 160 mg/dl
- Fasting plasma glucose ≥ 110 mg/dl
- History of anti-diabetic medication prescribed by a physician

Data Collection and Laboratory Analysis

After obtaining informed written consent, demographic and clinical data were collected using a pre-tested structured questionnaire. Venous blood samples were collected under aseptic conditions.

Biochemical parameters were analyzed using a fully automated analyzer:-

- **Plasma blood glucose:** GOD-POD method (Reference range: 70-160 mg/dl)
- **Serum calcium:** Arsenazo III method (Reference range: 8.9-10.3 mg/dl)
- **Serum phosphate:** Ammonium Molybdate method (Reference range: 2.5-4.5 mg/dl)

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using MS Excel and GraphPad software. Results were expressed as mean ± standard deviation (SD). Comparison between cases and controls was performed using the unpaired *t*-test. A p-value < 0.001 was considered statistically highly significant.

Ethical Considerations

Written informed consent was obtained from all participants, and confidentiality of data was strictly maintained.

Results

A total of 60 participants were included in the study, comprising 30 patients diagnosed with type 2 diabetes mellitus (cases) and 30 age- and gender-matched apparently healthy non-diabetic individuals (controls). A total of 60 participants were included in the study, comprising 30 patients with type 2 diabetes mellitus and 30 age- and gender-matched non-diabetic controls. There was no statistically significant difference in gender distribution between cases and controls (p>0.05).

The age of participants ranged from 30 to above 80 years. Most participants in both groups belonged to the 41-50 years age group. No statistically significant difference was observed in age distribution between the two groups (p>0.05), indicating that cases and controls were comparable with respect to age and gender (Table 1).

Table 1: Demographic characteristics of study participants, values expressed as number (%).

Variable	Controls (N=30)	T2DM Cases (N=30)	Total (N=60)	P-Value
Gender				
Male	13 (43.3%)	10 (33.3%)	23 (38.3%)	0.425
Female	17 (56.7%)	20 (66.7%)	37 (61.7%)	0.423
Age Group (years)				
30-40	5 (16.7%)	4 (13.3%)	9 (15.0%)	0.718
41-50	10 (33.3%)	15 (50.0%)	25 (41.7%)	0.190
51-60	6 (20.0%)	5 (16.7%)	11 (18.3%)	0.741
61-70	5 (16.7%)	3 (10.0%)	8 (13.3%)	0.447
71-80	3 (10.0%)	2 (6.7%)	5 (8.3%)	0.638
>80	1 (3.3%)	1 (3.3%)	2 (3.3%)	1.000

A significantly higher proportion of patients with type 2 diabetes mellitus exhibited decreased serum phosphate levels compared to non-diabetic controls (76.7% vs 10.0%; $p<0.001$). Similarly, decreased serum calcium levels were observed in 83.3% of diabetic patients as compared to 13.3% of controls ($p<0.001$). No statistically significant difference was observed in the proportion of participants with increased serum phosphate or calcium levels between the two groups (Table 2).

Table 2: Distribution of serum phosphate and serum calcium levels among cases and controls, values expressed as number (%).

Biochemical Parameter	Level (mg/dl)	Controls (N=30)	T2DM Cases (N=30)	P-Value
Serum Phosphate	Decreased (< 2.5)	3 (10.0%)	23 (76.7%)	< 0.001
	Normal (2.5-4.5)	25 (83.3%)	5 (16.7%)	< 0.001
	Increased (> 4.5)	2 (6.7%)	2 (6.7%)	1.000
Serum Calcium	Decreased (< 8.9)	4 (13.3%)	25 (83.3%)	< 0.001
	Normal (8.9-10.3)	23 (76.7%)	4 (13.3%)	< 0.001
	Increased (> 10.3)	3 (10.0%)	1 (3.3%)	0.298

The biochemical parameters of diabetic patients and controls are summarized in Table 3.

Table 3: Comparison of biochemical parameters between T2DM patients and controls

Parameter	T2DM Patients (Mean ± SD)	Controls (Mean ± SD)	P-Value
Plasma Blood Glucose (mg/dl)	212.5±30.4	106.4±43.8	< 0.001
Serum Phosphate (mg/dl)	2.2±0.56	3.07±0.21	< 0.001
Serum Calcium (mg/dl)	8.03±0.3	8.6±0.3	< 0.001

Plasma Blood Glucose

The mean plasma blood glucose level was significantly higher among patients with type 2 diabetes mellitus (212.5±30.4 mg/dl) compared to the control group (106.4±43.8 mg/dl). This difference was found to be statistically significant ($p<0.001$), confirming the hyperglycemic status of the diabetic group.

Serum Phosphate Levels

Serum phosphate levels were markedly reduced in patients with type 2 diabetes mellitus. The mean serum phosphate concentration among diabetic patients was 2.2±0.56 mg/dl, whereas in the control group it was 3.07±0.21 mg/dl. The observed difference between the two groups was statistically significant ($p<0.001$), indicating a strong association between diabetes mellitus and hypophosphatemia.

Serum Calcium Levels

Similarly, mean serum calcium levels were lower in the diabetic group (8.03±0.3 mg/dl) when compared to non-diabetic controls (8.6±0.3 mg/dl). This reduction in serum calcium levels among patients with type 2 diabetes mellitus was also found to be statistically significant ($p<0.001$), suggesting the presence of hypocalcaemia in the diabetic population.

Table 4: Effect Size (Cohen's *d*) of Biochemical parameters between T2DM Patients and Controls

Parameter	Cohen's *d*	Magnitude of Effect
Plasma Blood Glucose	2.98	Very large
Serum Phosphate	1.99	Large
Serum Calcium	1.90	Large

Effect size analysis using Cohen's *d* was performed to assess the magnitude of differences in biochemical parameters between patients with type 2 diabetes mellitus and non-diabetic controls (Table 2). Plasma blood glucose showed a very large effect size (D=2.98), indicating a substantial difference between the two groups. Serum phosphate levels demonstrated a large effect size (D=1.99), while serum calcium levels also showed a large effect size (D=1.90). These findings suggest that the observed differences in serum phosphate and calcium levels between cases and controls are not only statistically significant but also clinically meaningful.

Discussion

The present study demonstrates a significant reduction in serum calcium and phosphate levels in patients with type 2 diabetes mellitus compared to non-diabetic controls. These findings highlight the association between poor glycemic control and disturbances in mineral metabolism, which may contribute to the progression of diabetes and its complications.

Hyperglycemia induces osmotic diuresis, leading to increased urinary loss of phosphate. Phosphate excretion is directly influenced by blood glucose levels, and persistent hyperglycemia results in whole-body phosphate depletion. Reduced intracellular phosphate levels impair glucose phosphorylation and utilization, thereby exacerbating insulin resistance. Additionally, decreased muscle mass commonly observed in chronic diabetes further contributes to phosphate depletion [7].

Calcium plays a crucial role in insulin secretion from pancreatic β-cells. A reduction in serum calcium levels may impair insulin release and worsen hyperglycemia. Altered vitamin D metabolism, renal calcium loss, and decreased insulin action are possible mechanisms contributing to hypocalcaemia in diabetic patients [8].

The findings of the present study are consistent with those reported by Revathi *et al*., who demonstrated significantly lower serum phosphate levels in patients with type 2 diabetes mellitus.[7] Similarly, Kanchana *et al*. reported reduced serum calcium levels among diabetic patients, supporting the association between calcium metabolism and diabetes [8]. However, Nasir Abdelrafie Hamad *et al*. observed a significant reduction in serum phosphate levels without a corresponding change in serum calcium levels, suggesting possible population-based or methodological variations [9].

Variations in findings across studies may be attributed to differences in dietary intake, duration of diabetes, glycemic control, renal function, and study design. Nonetheless, available evidence suggests that disturbances in calcium and phosphate metabolism are common in patients with type 2 diabetes mellitus and warrant clinical attention.

The findings of the present study are consistent with previous studies that reported lower serum phosphate and calcium levels in T2DM patients. However, variations in results across studies may be attributed to differences in dietary intake, duration of

diabetes, glycemic control, and renal function.

In addition to statistical significance, the present study evaluated the magnitude of differences between patients with type 2 diabetes mellitus and non-diabetic controls using effect size analysis (Cohen's *d*). Effect size provides an estimate of the clinical importance of observed differences, independent of sample size, and is increasingly recommended in biomedical research.

Our study findings emphasize that disturbances in calcium and phosphate metabolism are substantial in patients with type 2 diabetes mellitus. Although these studies primarily focused on statistical significance, the magnitude of differences reported supports the large effect sizes observed in the present study [7, 8].

The assessment of effect size adds strength to the present study by demonstrating that alterations in serum calcium and phosphate levels in type 2 diabetes mellitus are not trivial findings. Instead, they represent clinically relevant biochemical disturbances that may contribute to insulin resistance, poor glycemic control, and long-term complications if left unrecognized and untreated. So, early detection and correction of calcium and phosphate imbalance may help improve insulin sensitivity, glycemic control, and prevent long-term complications such as osteoporosis and cardiovascular disease in diabetic patients.

Conclusion

The present study concludes that patients with type 2 diabetes mellitus have significantly lower serum calcium and phosphate levels compared to non-diabetic individuals. Hypocalcaemia and hypophosphatemia are significantly associated with hyperglycemia and may adversely affect insulin secretion and glucose metabolism. Routine monitoring of these electrolytes and timely correction through dietary modification or supplementation, along with standard diabetic care, may contribute to improved glycemic control and better clinical outcomes.

Recommendations

- Serum calcium and phosphate levels should be routinely assessed in patients with type 2 diabetes mellitus as part of comprehensive metabolic evaluation.
- Early detection and correction of hypocalcaemia and hypophosphatemia may help improve glycemic control and prevent metabolic complications in diabetic patients.
- Periodic monitoring of mineral status should be considered, especially in patients with poor glycemic control or long-standing diabetes.
- Further large-scale and longitudinal studies are recommended to confirm these findings and evaluate the role of mineral supplementation in diabetes management.

Source of Funding

Nil

Conflict of Interest

Nil

Authors' Contributions

- Dr. Rahima R Malek conceptualized and designed the study, laboratory investigations, supervised data collection, and contributed to manuscript drafting and critical revision.
- Dr. Sohil Mansuri was involved in data collection, statistical analysis, and preparation of the initial manuscript draft.
- Dr. Jayna Devalia contributed to data interpretation, literature review, and manuscript editing.
- All authors reviewed and approved the final manuscript.

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