Original Article

DISTURBANCES IN SERUM CALCIUM AND PHOSPHATE LEVELS IN PRE-DIABETES AND TYPE 2 DIABETES MELLITUS

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ABSTRACT

Objective: To evaluate serum calcium and phosphate levels in Pre-diabetic, Diabetic & non-diabetic subjects.

Material and Methods: This comparative cross-sectional study was conducted at the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, from July to December 2021. The study used a non-probability consecutive sample to include 300 people. Those people have been divided into three groups; diabetics, pre-diabetics and non-diabetics with a hundred people in every group. Blood specimens had been taken for the estimation of calcium, phosphate, HbA1c, plasma glucose and insulin in fasting in their respective tubes. SPSS version 24 was used to inspect the data. All descriptive variables had their means and standard deviations calculated. To examine descriptive characteristics within and between all groups, one way ANOVA and post hoc LSD were performed, and p-values 0. 05 were considered significant.

Results: Calcium and Phosphorus levels were lower in Diabetic group (Ca= 1.97±0.16mmol/l, PO_4 = 0.76±0.07 mmol/l) as compared to pre-diabetic group (Ca= 2.15±0.18 mmol/l, PO_4 = 0.93±0.06 mmol/l) and higher in non-diabetic group (Ca= 2.33±0.10mmol/l, PO_4 = 1.19±0.20 mmol/l) than both other groups. A one-way ANOVA and Post Hoc LSD revealed that the difference between and within groups was statistically significant with a p value of 0.01.

Conclusion: Our research has shown that as glycemic control slowly deteriorates, blood calcium and phosphate levels fall.

Key Words: Calcium, Phosphorus, Pre-diabetes, Type 2 diabetes mellitus.

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INTRODUCTION

Diabetes mellitus (DM) owing to its high prevalence has currently become a life-style globally. Diabetes mellitus (DM) is a diverse group of metabolic and hormonal illnesses in which the body is unable to properly digest and utilize the glucose in your diet [1]. It is characterized by the impaired glucose production or impaired response to insulin resulting in atypical carbohydrate metabolism and increased levels of blood glucose levels (hyperglycemia) [2]. According to a recent poll in Pakistan (2nd National Diabetes Survey of Pakistan), approximately 26.3% of people over the age of 19 have diabetes [3]. Of this population, 19.2% are known diabetics while 7.1% are newly diagnosed diabetics [4].

Diabetes mellitus Type 1 (T1DM) and Type 2 (T2DM) are the two primary kinds of DM [5]. T1DM is associated with HLA antigens and has weaker genetic link, while T2DM has stronger genetic link but exact genes are still unknown [6]. T2DM can result from obesity, physical inactivity and stress associated with insulin deficiency, insulin resistance or both [7]. Among all the diabetes patients, the prevalence of T2DM is 90% while rest of the 10% constitutes the other subtypes of DM [8]. Talking about Pakistan, the prevalence of T2DM mellitus has reached up to 16.96% [9].

It is known that disturbances in calcium (Ca) levels can affect the secretion of insulin from pancreatic β cells [10]. This is due to the fact that the glucose-dependent insulin secretion is regulated by Ca which depends upon its concentrations in pancreatic β cells [11]. Ca influx through voltage-gated Ca channels is necessary for the release of insulin from pancreatic beta cells [12]. It was known that the function of pancreatic β cells is associated with the serum Ca levels. Alterations in the Ca influx can result in the abnormal β-cell function and consequently can increase the risk of T2DM [13]. Also, the disturbed Ca levels can reduce the expression of GLUT 4 transporters; hence the uptake of glucose will be reduced causing high plasma glucose levels [14].

Phosphate (PO4) is similarly required for the synthesis of ATP, which provides energy for the cells [15]. Insulin resistance and poor glucose tolerance

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can emerge from interferences in the energy metabolism caused by low serum PO4 levels [16]. Low serum PO4 levels prevent the phosphorylation of intermediates made of carbohydrates, which results in hyperglycemia [17]. Ca and PO4 are vital minerals that have extensive position in energy metabolism. Insulin resistance and glucose tolerance impairment have significant effects on energy metabolism, mineral activities, as well as Ca and PO4. To our knowledge no local data is available showing effects of impaired glucose tolerance and T2DM on Ca and PO_4 metabolism. The purpose of this study was to assess the changes in blood Ca and PO4 levels in patients with T2DM and impaired glucose tolerance (Pre-Diabetes) which may help to prevent. This will help early detection and treatment may help reduce complications related to abnormalities of these elements.

MATERIAL AND METHODS

This comparative cross-sectional study was performed at department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology Rawalpindi from July 2021 to December 2021. The study was conducted after Institutional Ethical Review board approval. All participants provided their written, informed permission in the form of consent performa. Three hundred gender matched individuals aged more than 20 years with T2DM, Pre-diabetes and healthy participants had been included in this study. Those with bone mineral disorders, chronic kidney and liver diseases, cancer, and drug use that interfered with the metabolism of Ca and PO4 were not included in the study. The WHO calculator was used to determine the sample size with prevalence 26.3% (3), confidence interval of 95% and error margin of 5%. The selected individuals were divided into three groups; T2DM, Pre-diabetics and Non-diabetics with 100 individuals in each. A structured study proforma was used to gather the data. Blood was drawn into gel tubes to measure albumin-corrected total calcium, total phosphorus, and fasting insulin; EDTA tubes to measure HbA1c; and sodium fluoride tubes to measure fasting plasma glucose. Fasting insulin levels were measured on immunoassay auto analyzer Immulite 2000, while the rest of the parameters were measured on clinical chemistry auto analyzer ADVIA 1800 by their respective methods.

The statistical programme for social sciences (SPSS) version 24 was used to enter and analyze the data. The quantitative data was expressed as mean and standard deviation (SD). Analysis of variance (One way ANOVA) was performed to compare characteristics of the three groups both within and between them, and the Post Hoc LSD test was employed for numerous comparisons. A p-value of 0.05 was regarded as statistically significant.

RESULTS

Out of the 300 people that took part in the study, 177 (59%) were men (males) and 123 (41%) were women (females). The individuals' average age was 43.77. Participants were separated into three groups. Diabetics, Pre-diabetics, and Non-diabetics and Table-I displays the descriptive statistics for all variables in each category.

Calcium and Phosphorus levels were lower in Diabetic group (Ca= 1.97±0.16mmol/l, PO_4 = 0.76±0.07 mmol/l) as compared to pre-diabetic groups (Ca= 2.15±0.18 mmol/l, PO_4 = 0.93±0.06 mmol/l) and higher in non-diabetic group (Ca= 2.33±0.10mmol/l, PO_4 = 1.19±0.20 mmol/l) than both other groups as shown in Figure-I.

One-way analysis of variance (ANOVA) was used to compare the mean serum total Ca and PO4 levels in the diabetic group to those in the pre-diabetic group and those in the non-diabetic group. Table-II demonstrates differences within and between groups that were statistically significant (p value 0.05). Multiple comparisons by Post Hoc LSD test also showed that difference was statistically significant between all the three groups in all variables (*p* value <0.05) except in fasting serum Insulin levels between diabetic and pre-diabetic groups (*p*-value=0.29) as shown in Table-III.

Table-I: Descriptive statistics of all the variables in diabetic, non-diabetic & pre-diabetic individuals (n=100).

Variables	Diabetic group Mean±SD	Pre-diabetic group Mean±SD	Non-diabetic group Mean±SD
HbA1c	8.31±1.6	6.5±5.3	5.15±0.29
FPG	7.21±1.9	5.81±0.59	4.7±0.53
FI	17.2±12.4	15.28±8.30	11.72±5.67
HOMA-IR	5.38±4.02	4.06±2.71	2.47±1.26
Total Ca	1.97±0.16	2.15±0.18	2.33±0.10
PO_4	0.76±0.07	0.93±0.06	1.19±0.20

Note: HbA1c= Glycosylated hemoglobin, FPG= Fasting Plasma Glucose, FI= fasting insulin HOMA-IR= Homeostatic Model Assessment for Insulin Resistance, Ca= Calcium. PO_4=Phosphate

Table-II: Results of One Way ANOVA of all the variables in diabetic, non-diabetic and pre-diabetic groups.

		Sum of squares	Mean squares	F	*P*-value
HbA1c	Between groups	499.59	249.79	24.17	<0.01
	Within groups	3069.33	10.33		
FPG	Between groups	295.27	147.63	98.15	<0.01
	Within groups	446.67	1.50		
FI	Between groups	1567.68	783.84	9.14	<0.01
	Within groups	25461.81	85.73		
HOMA-IR	Between groups	423.88	211.94	25.20	<0.01
	Within groups	2497.66	8.41		
Total Ca	Between groups	6.59	3.29	299.68	<0.01
	Within groups	3.26	0.01		
PO_4	Between groups	9.37	4.68	265.24	<0.01
	Within groups	5.24	0.01		

Note: HbA1c= Glycosylated hemoglobin, FPG= Fasting Plasma Glucose, FI= fasting insulin HOMA-IR= Homeostatic Model Assessment for Insulin Resistance, Ca= Calcium. PO_4=Phosphate

Table-III: Multiple comparison result of Post Hoc LSD ANOVA for all the variables in Diabetic, Non-diabetic and pre-diabetic groups.

Dependent variable	Diabetic status (I)	Diabetic status (J)	Mean difference	Standard error	*p*-value
HbA1c	Diabetic	Non-diabetic	3.15	0.45	<0.01
		Pre-diabetic	1.74	0.45	<0.01
	Non-diabetic	Pre-diabetic	-1.40	0.45	<0.05
FPG	Diabetic	Non-diabetic	2.42	0.17	<0.01
		Pre-diabetic	1.40	0.17	<0.01
	Non-diabetic	Pre-diabetic	-1.01	0.17	<0.01
FI	Diabetic	Non-diabetic	5.52	1.30	<0.01
		Pre-diabetic	1.96	1.30	0.29
	Non-diabetic	Pre-diabetic	-3.56	1.30	<0.05
HOMA-IR	Diabetic	Non-diabetic	2.90	0.41	<0.01
		Pre-diabetic	1.32	0.41	<0.05
	Non-diabetic	Pre-diabetic	-1.58	0.41	<0.01
Total Ca	Diabetic	Non-diabetic	-0.36	0.01	<0.01
		Pre-diabetic	-0.18	0.01	<0.01
	Non-diabetic	Pre-diabetic	0.17	0.01	<0.01
PO_4	Diabetic	Non-diabetic	-0.42	0.01	<0.01
		Pre-diabetic	-0.16	0.01	<0.01
	Non-diabetic	Pre-diabetic	0.26	0.01	<0.01

Note: HbA1c= Glycosylated hemoglobin, FPG= Fasting Plasma Glucose, FI= fasting insulin HOMA-IR= Homeostatic Model Assessment for Insulin Resistance, Ca= Calcium. PO_4=Phosphate

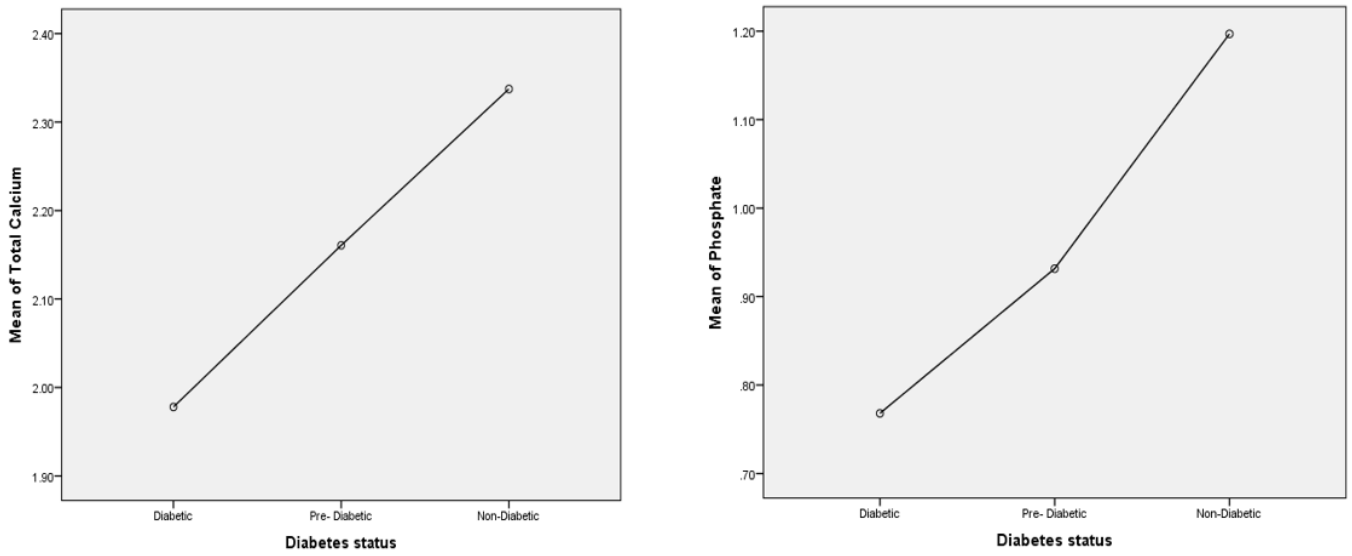

Figure-I: Graphical presentation of mean calcium and phosphate levels in diabetic, pre-diabetic and healthy subjects.

DISCUSSION

Ca and PO_4 are two important minerals that take part in many important biological functions. Disturbances in the levels of these two minerals have multiple effects on our body. The insulin release from pancreatic beta cells is regulated by Ca, while PO_4 has important role in energy metabolism. In DM the disturbances in levels of Ca and PO_4 may cause poor

glycemic control and worsening of its complications due to interferences in the energy metabolism resulting in impaired insulin sensitivity and glucose tolerance impairment. As no local data was available, we conducted this study to evaluate the disturbances in serum Ca and PO_4 levels in abnormal subjects having glucose tolerance impairment (Pre-Diabetes) and T2DM as compared to healthy subjects.

The study's participants were separated into three categories: pre-diabetics, diabetics, and non-diabetics. In order to compare the concentrations of total calcium and inorganic phosphate in these groups, one way analysis of variance (ANOVA) was used. The levels of Ca and PO4 were greater in the non-diabetic group (Ca= 2.330.10mmol/l, PO4 = 1.190.20 mmol/l) than in the diabetic group (Ca= 1.970.16mmol/l, PO4 = 0.760.07 mmol/l) and pre-diabetic group (Ca= 2.15±0.18 mmol/l, PO4 = 0.93±0.06 mmol/l) and the difference between the groups and within the groups was statistically significant with a p value of 0.01. Multiple comparison by Post Hoc LSD test also showed that difference was significant between all the three groups in all variables (*p*-value <0.05) except between diabetic and pre-diabetic group in Fasting Insulin (*p*-value=0.29).

Sohan Lal Nigah *et al.* in 2020 in India reported similar results as in our study. They included 150 T2DM patients and 150 healthy individuals as controls. Ca and PO4 levels were lesser in T2DM patients compared to healthy individuals (p value 0.05) [18]. In 2020, Lata Kanyal *et al.* conducted a study on 100 patients of T2DM and 100 healthy subjects of same age and gender. Same as our study, the diabetic group's Ca and PO4 levels were lower than those of the controls [19]. Regardless of age, gender, or body fat percentage, M. Haap *et al*. (2006) discovered an inverse relationship between serum PO4 and 2-hour glucose levels (r=0.13, P0.0001). Less blood PO4 levels at baseline in the research participants were associated with higher postprandial glucose levels. They came to the conclusion that lower serum PO4 levels are associated with higher 2-hour glucose levels and decreased insulin sensitivity in non-diabetic patients. Future research must examine whether the low serum PO4 levels are the root cause or effect of impaired glucose tolerance and low insulin sensitivity [20]. Our results also showed that the serum phosphorus levels decrease as the glucose intolerance increases.

Nasir Hamad *et al.* in 2012 reported that no statistical difference was found in Ca levels between T2DM patients and controls which was different compared from our results. However, levels of phosphorus were low among diabetics as compared to healthy individuals (p value = 0.001) as reported in our results [21].

In 2021, Maryam Barghi and colleagues conducted a study at Kermanshah University of Medical Sciences' Imam Reza Hospital in Kermanshah, Iran. 40 T2DM patients and 40 healthy people (Controls) between the ages of 20 and 60 were included. The serum levels of Ca and PO4 in the diabetic and non-diabetic groups did not differ noticeably [22]. These results were at odds with what we found.

The difference in results of variant studies as compared to our study may be due to different sample size, different setting as well as population and methodology used to collect data and analytical method used. Since our study was conducted at just one location, it has certain limitations. This study was conducted with a small sample size and duration of study was short with no follow up. Future multicenter, case control studies may be conducted focusing on long term effects of supplementation and monitoring on glycemic control to further evaluate effects of Ca and PO_4 on T2DM.

CONCLUSION

Mean serum calcium and phosphate levels were lower in Diabetics and Pre-diabetics as compared non-diabetics. The levels decrease with the worsening of glycemic control. Supplementation of these important minerals along with monitoring may improve glycemic control and delay complications

AUTHOR CONTRIBUTION

Tehreem Fatima Awan: Project development, Data analysis, interpretation of results, literature search, critical reviews.
Muhammad Anwar: Project conception, interpretation of results, literature search, critical reviews.
Zujaja Hina Haroon: data collection and processing, analysis and/or, literature search manuscript writing.
Afshan Bibi: Data collection and processing, literature search, manuscript writing, critical reviews.
Muhammad Younas: Data collection and processing, literature search, manuscript writing, critical reviews.
Sobia Irum Kirmani: Data collection and processing, literature search, manuscript writing, critical reviews.

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