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"CORRELATION OF SERUM CALCIUM, PHOSPHORUS AND VITAMIN-D IN TYPE-2 DIABETIC MELLITUS PATIENTS ATTENDING RAMA MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE KANPUR".

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ABSTRACT

BACKGROUND: Type-2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder characterized by insulin resistance and hyperglycemia. Emerging evidence suggests that mineral metabolism, particularly involving calcium, phosphorus, and vitamin D, may play a crucial role in the pathophysiology of T2DM.

AIM: To study the Correlation of Serum Calcium, Phosphorus and Vitamin-D in Type-2 Diabetic Mellitus patients Attending Rama Medical College Hospital & Research Centre Kanpur.

MATERIAL & METHODS: This was a Hospital Based Prospective Cross-sectional between case and control group carried out for a period of twelve month in the Department of Biochemistry Rama Medical College Hospital & Research Centre Kanpur. A total of 80 patients were assessed out of which 40 were healthy individuals (control group) and other 40 were individulas with type-2 diabetes mellitus patients (cases) . Blood samples were collected after overnight fasting under all aseptic conditions for fasting plasma glucose level, postprandial glucose level, glycated hemoglobin, vitamin-D, calcium and phosphorous level were studied.

RESULTS: This study include 80 individuals which was divided into two groups. Group I(CASE GROUP): include diabetic patients (n=40), in which males were 24(60%) and females were 16(40%). The maximum number of diabetic patients were found in the age group of 61-

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70 (30%) .In diabetic patients, 31(77.5%) individuals have phosphorous level recorded with <3.4mg/dl. 15(37.5%) individuals have serum calcium level less than 8.4mg/dl and only 22.5% of diabetic patients were observed to be less concentration of Vitamin-D level.

CONCLUSION: Monitoring serum levels of calcium, phosphorus, and vitamin D is crucial for tracking the prognosis of type 2 diabetes. These are the early parameters and should be considered in the diagnosis of type-2 diabetes mellitus.

KEYWORDS: Type-2 Diabetes mellitus, serum calcium, phosphorous, vitamin-D level, glycemic index.

INTRODUCTION

The incidence of diabetes mellitus Type 2 (T2DM) is increasing at an alarming rate; the significant increase in the number of people with diabetes will be in India [1]. It is the most common metabolic disease. It was initially reported 3000 years ago in Egyptian manuscripts and the distinction between type 1 and type 2 diabetes mellitus was established in 1936 [2,3] .

Most of the expected population growth between 2000 and 2030 will be concentrated in the urban areas of the world [1]. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 [4].

Diabetes mellitus refers to a group of metabolic disorder with a multiple etiologies that are defined by chronic hyperglycemia and includes lipid, protein and carbohydrate metabolic abnormalities induced by insulin production and insulin action [5] .

Insulin, an anabolic hormone that causes metabolic irregularities in carbohydrate, lipids, minerals and a variety of others substances. Low insulin secretion, decrease glucose utilization and elevated glucose synthesis are all variables that contribute to hyperglycemia depending on etiology of diabetes mellitus [6,7] . According to the ICMR INDIAB India had 62.4 million diabetic patients in 2011 and is anticipated to have 101.2 million by 2030 [8] .

In developing countries, the majority of people with diabetes are in the age group of 45–64 years, in contrast: the majority of people with diabetes in developed countries are >64 years of age.[9] By 2030, it is estimated that the number of people with diabetes >64 years of age will be >82 million in developing countries and >48 million in developed countries [9].

The American Diabetes Association proposed by the classical categorization of type 1 diabetes mellitus, type 2 diabetes mellitus & gestational diabetes mellitus and it is the most often used classification by the ADA [10] .

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In 1988, type 2 diabetes mellitus was known as the primary component of metabolic syndrome. In 2011, 366 million people were expected to have diabetes mellitus by 2030 that number will have risen to 552 million [11, 12] .

Non-insulin-dependent diabetes mellitus, also known as type 2 diabetes is a condition with elevated blood glucose levels (hyperglycemia) insulin resistance and may combined with a deficiency of insulin [13] .

Diabetes mellitus 2 is the endocrine disease characterized by hyperglycemia due to progressive failure of pancreatic cells in a setting of chronic insulin resistance [14]. In experimental studies, calcium and Vitamin D have been shown to improve pancreatic beta-cell function and peripheral insulin sensitivity, whereas low Vitamin D status is associated with markers of impaired glucose metabolism [14,15] and insulin resistance [16] In recent decades, insulin resistance and secretion have been shown to depend on calcium and Vitamin D homeostasis [17] Insulin secretion is a calcium-dependent process requiring the influx of calcium to the beta-cell [17,18].

The phosphate lowering action of insulin was recognized immediately after insulin preparations became available [19] There is a close correlation between the plasma concentration of phosphorus and the degree of metabolic control of diabetes [20-24].

Therefore, the main aim of this was to analyze the serum calcium level, serum phosphorous level and vitamin -D level in type-2 diabetes mellitus patients and in healthy individuals, and also assess the correlation between these parameters.

MATERIAL & METHODS

This was a hospital based prospective cross-sectional between case and control group conducted in Department of Biochemistry, Rama Medical College Hospital & Research Centre Kanpur, UP.

A Total of 80 patients were included in this study and divided into two groups. The informed written consent was obtained from all the patients studied.

Group1: (CASE GROUP) consist of 40 patients with type -2 Diabetes mellitus.

Group 2: (CONTROL GROUP) consists of healthy individuals.

Inclusion criteria: Patients above aged above 20 years having type-2 diabetes mellitus.

Exclusion criteria: Patients with other complications like Chronic liver disease, Pregnant

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women, heavy smokers, patients taking drugs like isoniazid, rifampicin and patients on vitamin-D therapy were excluded from this study.

Ethical clearance: Ethical clearance was duly obtained from the Institutional Ethical Committee of RMCH&RC.

Sample collection: Under all the aseptic condition, 5ml blood sample was collected from antecubital vein after overnight fasting in both state(fasting and postprandial). A 3 ml of blood was taken in plane vial and allowed to clot to separate out serum then it was centrifuged for 10 mins at 3000 rpm and this centrifuged sample was used for the estimation of serum calcium, phosphorous and serum 25-hydroxyVitamin -D.

The other 2 ml of blood was poured in EDTA vial to investigate the fasting blood sugar level. Postprandial blood glucose level was measured 2 hours after the meal.

Serum 25(OH) D was estimated with the ELISA method [21]. Estimation of serum calcium was done with Arsenazo 111 method/ cresalphthalein complexions method [22]. Estimation of serum phosphorus with Molybdate U. V. method [23] FPG and 2-h PPPG were measured using enzymatic methods (GOD-POD) [24].

Statistical analysis: The data were analyzed with excel software version 12. Values of $P < 0.05$ were assumed to be significant ($P < 0.05$).Pearson correlation coefficients were calculated to assess the relationships between serum calcium, phosphorus, and vitamin D levels.

RESULTS

A total of 80 individuals which were divided into two groups were studied. Group I (CASE GROUP): include diabetic patients (n=40), in which males were 24(60%) and females were 16(40%). Their ages were observed between 20 to 80 years. (Table 1 and Table 2).

The maximum number of diabetic patients were found in the age group of 61-70 (30%) followed by 27.5% were found in the age group of 51-60 and only 5% of diabetic patients were found in the age group of 20-30. In group 2 (CONTROL GROUP): there were 35(87.5%)were females and 5(12.5%) were males. Among them the 10(25%) of individuals were belong to age group 61-70 while only 3 (7.5%) were belong to age group 31-40. (Table 1 and Table 2).

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Table 1: Comparison of Gender between healthy individuals and diabetic patients.

GENDER	**CONTROL GROUP(n=40)**	**DIABETIC PATIENTS(n=40)**
MALES	5(12.5%)	24(60%)
FEMALES	35(87.5%)	16(40%)

In this table it was observed that, 60% and 40% males and females were diabetic respectively.

Table 2: Comparison of Age between control group and diabetic patients.

AGE	**DIABETIC PATIENTS(n=40)**	**CONTROL GROUP(n=40)**
20-30	2(5%)	7(17.5%)
31-40	3(7.5%)	3(7.5%)
41-50	7(17.5%)	11(27.5%)
51-60	11(27.5%)	8(20%)
61-70	12(30%)	10(25%)
>71	5(12.5%)	1(2.5%)

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From the Table No. 2 it was observe that, maximum number of diabetic patients were from the age group between 40-70 years. This age group was also similar with the control group.

The serum phosphorous level was higher in diabetic patients than in healthy individuals. In diabetic patients, 31(77.5%) individuals have phosphorous level <3.4mg/dl while 8(20%) individuals have phosphorous level is in normal range between 3.5 to 4.5mg/dl and only one patient have phosphorous level > 4.5mg/dl. As shown in table 3.

Table 3: Comparison of Serum Phosphorous level between Diabetic patients and in control groups.

SERUM PHOSPHOROUS LEVEL	**DIABETIC PATIENTS(n=40)**	**CONTROL GROUP(n=40)**	**P Value**
<3.4mg/dl	31(77.5%)	5(12.5%)	0.059
3.4- 4.5mg/dl	8(20%)	32(80%)	
>4.5mg/dl	1(2.5%)	3(7.5%)	

In this table it was observed that, serum phosphorous level was less than normal range in diabetic patients which include 77.5% of individuals while 80% healthy individuals shows serum phosphorous level in normal range.

The serum calcium level in diabetic patients (n=40) was found to be less than the healthy individuals. Among diabetic patients, 15(37.5%) individuals have serum calcium level less

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than 8.4mg/dl. While 22(55%) individuals have serum calcium level is in between normal range which is 8.5-10.5mg/dl and only 3(7.5%) individuals have serum calcium level >10.5mg/dl. Comparison of serum calcium level between diabetic patients and healthy individuals is given in table 4.

Table 4: Comparison of Serum calcium level between diabetic patients and control group.

SERUM CALCIUM LEVEL	DIABETIC PATIENTS(n=40)	CONTROL GROUP(n=40)	P Value
<8.4mg/dl	15(37.5%)	0	0.017
8.5- 10.5mg/dl	22(55%)	40(100%)	
>10.5mg/dl	3(7.5%)	0	

In this table it was observed that, 100% healthy individuals have serum calcium level is in the normal range. While in diabetic group, 37.5% individuals shows calcium level <8.4mg/dl.

In this study it was observed that the deficiency of vitamin-D level in diabetic patients was quite low as only 9(22.5%) of diabetic patients shows vitamin-D deficiency.Comparison of vitamin-D level among healthy and diabetic patients was shown in Table 5.

Table 5: Comparison of Serum 25-hydroxy Vitamin-D level between diabetic patients and control group.

SERUM 25-hydroxy Vitamin-D level	DIABETIC PATIENTS(n=40)	CONTROL GROUP(n=40)	P value
<20ng/ml	9(22.5%)	0	0.017
20-40ng/ml	31(77.5%)	40(100%)	

In this table it was observed that, only 22.5% of diabetic patients were less concentration of Vitamin-D level.

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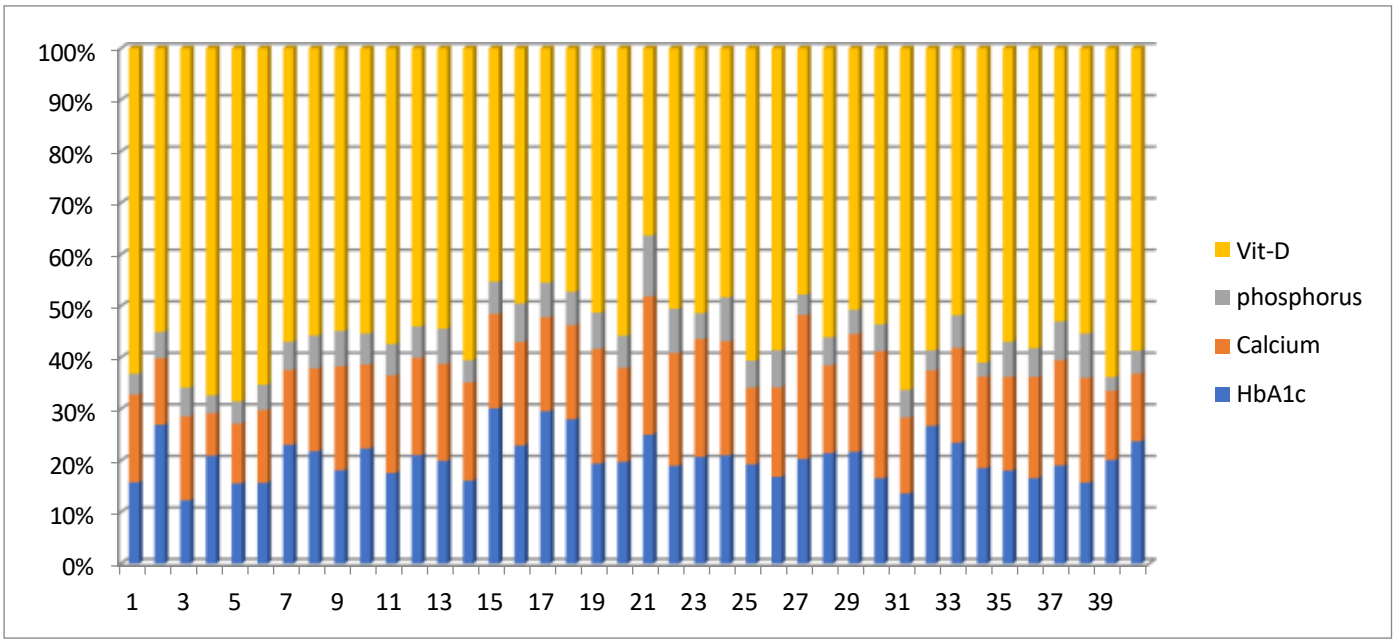
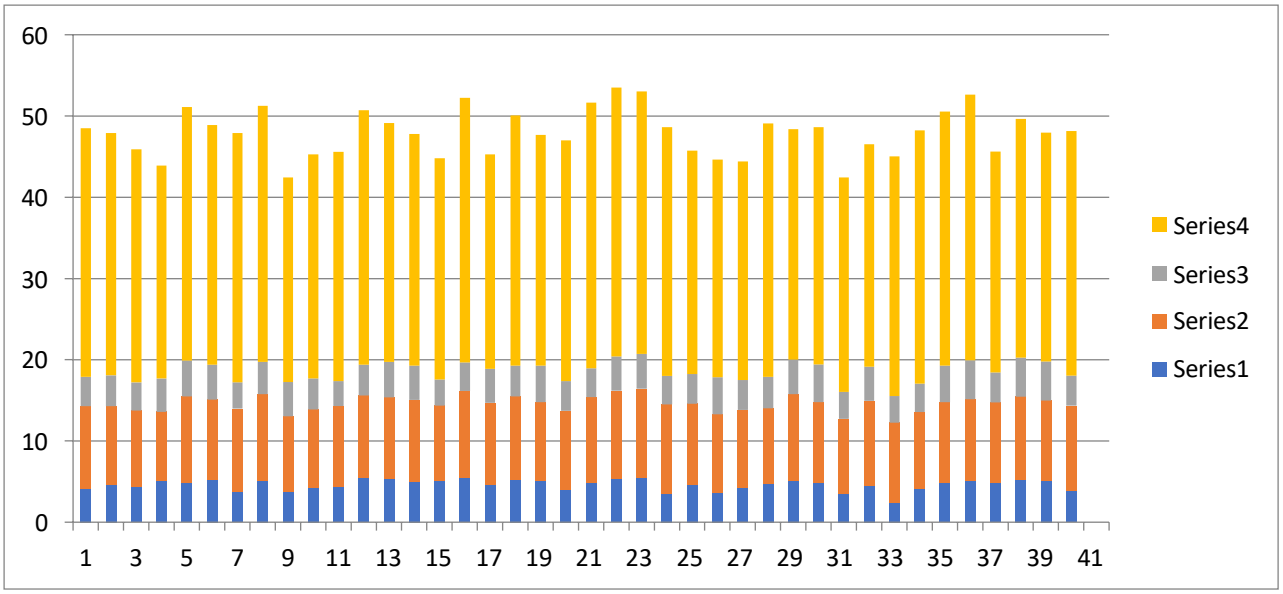


Graph: 1: Bar Chart showing frequency distribution of HbA1C, Seum Calcium, Phosphorus and Vit-D levels among Cases



Graph 2: Bar Chart showing frequency distribution of HbA1C, Seum Calcium, Phosphorus and Vit-D levels among Control group

In the current study the HbA1C versus Serum Calcium was studied where Chi ^{2} : 8.11, P-value: 0.017 which was observed to be statistically significant. The result for HbA1C versus

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Serum Phosphorus was found to be Chi ^{2} : 5.79, P-value: 0.059 which was noted to be statistically significant.

The result for HbA1C versus Serum Vit-D Chi ^{2} : 11.07, P-value: 0.017 which was observed to be statistically significant.

DISCUSSION

Diabetes mellitus is a metabolic disorder. It is a group and various degrees of carbohydrate, lipid and protein metabolism impairment [25] .

Diabetes mellitus can have a variety of causes and etiologies, but it usually involves aberrant insulin secretion & response at some point throughout the disease's evolution. Type 1 diabetes (autoimmune diabetes) and type 2 diabetes mellitus are the two most frequent types of diabetes mellitus both are characterized by hyperglycemia, insulin resistance and relative insulin shortage [13].

In our study the ratio of most diabetic patients were observed to be male (60%) as compared to the females (40%). The diabetes mellitus was seen most prevalent in the age group of 40-70 years. Our study was in correlated with the study conducted by other author Qadri et al in year 2017, where most patients were male and higher diabetic prevalence were seen in the age group of 30-60 years and patients suffering from diabetes mellitus female patients were about 22% of the total [26]. While study by Marwa *et al*. was in constrast to the present study where that prevalence of T2DM in females was found to be 63% than males, i.e., 37% [27].

In our study, it was observed that the comparison of age between control group and diabetic group were in the age group of 21-70 years. There were no significant changes in the age group of controls and case group.

In present study, we compared serum calcium level in control groups and diabetic patients, the serum calcium level in diabetic patients were low as compared to control groups (healthy individuals). There were 37.5% diabetic patients shows decreased in calcium level. This correlates with the study conducted by Qadri et al, where they recorded that the mean value of serum calcium (7.98±0.91 mg/dl) in diabetes patients were significantly (p<0.001) decreased when compared with prediabetes patients (8.53±0.84 mg/dl) [26].

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It was observed that that the serum phosphorous level in diabetic patients were significantly low as compared to healthy individuals, in our study, 77.5% diabetic patients were shown <3.4mg/dl serum phosphorous level. This study is significant with P-value: 0.059.($p<0.001$).

The study done by Kanchana N, *et al*. 2014 [28] observed that serum level of calcium with the mean value of (9.4±0.38 mg/dl) was significantly decreased in type 2 diabetes mellitus patients then controls and also found a negative correlation between fasting plasma glucose and serum calcium concentration, that prove increased glucose levels was associated with decreased calcium levels.

While study by Nigah, *et al*.Serum calcium levels in patients were found to be in the range of 7.20–11.40 mg/dl, while the range of serum calcium concentration in the healthy individuals under study was 7.0–10.80 mg/dl. Thus, serum calcium concentration was significantly lower in patients as compared to controls [29].

Serum phosphorus levels in patients were found to be in the range of 2.8–6.0 mg/dl, whereas the range of serum phosphorus concentration in the healthy individuals understudy was 2.4–10.80 mg/dl. Thus, serum phosphorus concentration was also comparatively lower in patients as compared to controls with *P* value 0.001 at ($P < 0.05$) [29] and was observed to be statistically significant.

In our study, the vitamin-D level in both diabetic group and control group were in the normal range. There is no correlation between vitamin -D and type-2 diabetes mellitus. The studies conducted by Nigah et al[29] showed that Vitamin D deficiency was more frequent in T2DM females 79.7% than males 71.2% this finding confirmed by Arif *et al*. 2017 who reported that T2DM female were four times more prone for Vitamin D deficiency than males 151 (79%) and 40 (21%), respectively.[30] Vitamin D levels of patients were found to be in the range of 2.25–55.13 while that of controls were in the range of 1.51–71.0 ng/ml which slightly varies from a study conducted by Bayani *et al*., in which mean concentration of Vitamin D in the case group was 18.7 ± 10.2 and in the control group was 24.6 ± 13.5 ng/dl.[31]

This study concluded that, there is a significant correlation in serum calcium, serum phosphorous and vitamin-D level among diabetic patients and control groups. Hence, these parameters should be considered in early detection of type-2 diabetes mellitus among individuals.

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CONCLUSION

The substantial relationships between vitamin D, calcium, and phosphorus in serum and people with type 2 diabetes are made apparent by this study. According to these results, treating vitamin D insufficiency and making sure that mineral metabolism is balanced may be helpful in the overall management of type 2 diabetes. It is necessary to do additional longitudinal study to investigate the underlying mechanisms and related linkages as the decreasing levels of serum Vitamin D , calcium, and phosphorus may influence glycemia.

Declarations:

Conflicts of interest: There is no any conflict of interest associated with this study

Consent to participate: We have consent to participate.

Consent for publication: We have consent for the publication of this paper.

Authors' contributions: All the authors equally contributed the work.

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