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Original Research Article

ASSESSMENT OF VITAMIN D AND CALCIUM LEVELS IN TYPE 2 DIABETES MELLITUS

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Abstract: *Diabetes mellitus is an endocrinological condition marked by poor carbohydrate metabolism. T2DM is increasing both domestically and globally. Vitamin D and calcium have a significant role in intermediate metabolic processes, which include enzyme activity, electrical gradients, and the functioning of cells. T2DM was shown to be correlated with abnormal calcium and vitamin D levels. The objective of the current study is to assess the calcium and vitamin D levels in 200 patients, of whom 100 were T2DM patients and 100 were healthy, non-diabetic controls. 12 hours of entire night fasting, a volume of 5 ml of blood was drawn completely aseptically from the antecubital vein to measure glycated hemoglobin, fasting plasma glucose (FPG), postprandial plasma glucose (PPPG), serum 25 hydroxy (OH) Vitamin D (25[OH]D) and calcium. There was a statistically significant difference between the type 2 diabetes and non-diabetic groups for the following measurements: FPG (P 0.001), PPPG (P 0.001), serum vitamin D (P 0.001), and calcium (P 0.001). Comparing T2DM patients to the control group, lower calcium and vitamin D levels were found. This study found that Type 2 diabetes patients had much lower vitamin D and calcium levels, which may play a key role in the disease's propensity to develop early in vulnerable people.*

Keywords: 25 Hydroxy Vitamin D, Calcium, Type 2 Diabetes Mellitus

Introduction

The prevalence of Type 2 diabetes mellitus (T2DM) is rising dramatically. According to calculations, there will be 366 million people with diabetes worldwide by 2030, up from 171 million in 2000 (Dubey et al.). Almost all diabetics in developing nations are between the ages of 45 and 64; in contrast, the majority of people with diabetes in advanced countries are over the age of 64 (Tachkov et al., 2020). In developing countries, there will be over 82 million diabetics over 64 by 2030, compared to over 48 million in advanced nations. With an estimated incidence rate of 6.9%, Pakistan had 7.5 million cases of diabetes diagnosed in 2017 (Abdulghani et al., 2021). The number of diabetes patients has grown dramatically over the past several years, and this is thought to be one of the biggest threats to the nation's healthcare system. We must not only upgrade the medical facilities but also look at the many variables that affect our people (Kopitar et al., 2020). There are two primary kinds of diabetes mellitus: type I (T1DM), also known as insulin deficient diabetes, occurs when the pancreatic islet cells that secrete insulin are unable to produce adequate insulin to keep blood sugar levels within the normal range for the body (90–180 mg/dl) (Eizirik et al., 2020). Diabetes II (T2DM), sometimes referred to as insulin-resistant diabetes, is the second most prevalent kind (Kostov, 2019). Low Vitamin D status is linked to signs of poor glucose metabolism and insulin resistance, whereas calcium and Vitamin D have been proven in experimental investigations to increase pancreatic beta-cell activity and peripheral insulin sensitivity (Greco et al., 2019). Recent research has shown that calcium and vitamin D affect insulin resistance and secretion (Contreras-Bolívar et al., 2021; Greco et al., 2019; Szymczak-Pajor and Śliwińska, 2019). A calcium influx into the beta cell is necessary to produce insulin. The biggest concern is whether diabetes affects calcium and vitamin D levels (Zhang et al., 2022). Therefore, the major goal of this study is to determine if diabetics and non-diabetics have different levels of blood Vitamin D and serum calcium, as well as the relationship between these levels and other biochemical markers in the two groups.

Methodology

The current study was a comparative case-control study in the Department of Medicine at Sir Ganga Ram Hospital, Lahore, from March 2018 to March 2019. One hundred documented cases of T2DM in the 30 to 60-year age range and 100 healthy people were chosen as the study control group. After receiving institutional clearance from the ethics committee, this study was carried out, and written consent was obtained from all the study participants.
Patients with T2DM also had additional comorbidities, including chronic liver disease or chronic renal disease, taking medications like rifampicin, isoniazid, ketoconazole, or phenytoin, or receiving vitamin D treatment were not included in the research. Patients with hypertension and expectant mothers were removed as well from the trial.
5 ml of blood from the venous system was drawn out under aseptic conditions when the subject was fasting and after eating. A fresh, sterilized vial with 3 ml of blood was filled, and it was left to coagulate for 30 minutes at room temperature. Serum was filtered by centrifugation at 3000 spins per minute for 10 minutes, and it was used to measure serum 25[OH] Vitamin D and serum calcium. 2 cc of blood was placed into a sugar vial containing an anticoagulant (sodium fluoride and potassium oxalate) to investigate the fasting plasma glucose (FPG). Additionally, postprandial plasma glucose (PPPG) was assessed using standard kits

two hours after meals (75 g of glucose in a glass of water). The ELISA technique was used to estimate serum 25(OH) D levels. Cresalphthalein complexions and the Arsenazo 111 technique were used to estimate the serum calcium levels. Enzymatic techniques assessed the FPG and 2h PPPG (GOD-POD). Height and weight measurements were taken, and the body mass index (BMI) was computed. The mean ± standard deviation (SD) was used to represent the data. The statistical significance of any baseline differences among groups was assessed using the independent t-test, and Pearson correlation was computed using the expression. Data were examined using SPSS version 12 software. P < 0.05 values were considered significant (P 0.05).

Results

100 cases of diabetes (n = 100) and 100 normal subjects (n = 100) were used in this case-control research. The research population's (n=200) demographic characteristics are summarized in [Table 1]. 55.0 percent of the participants in the case group were females, 47% in the control group were women, and the bulk of total participants were between the Ages of 51 and 60. The findings revealed no differences in sex, age, region, or BMI amongst cases and control group. The findings were presented as mean± SD. Age, BMI, FPG, PPPG, glycated hemoglobin (HbA1c), serum Vitamin D, and calcium all showed in statistical terms significant differences (P <0.001) among the two groups(Table 2). Between people with diabetes, male and female, the findings indicated a statistically significant disparity (P <0.05) in BMI and no significant difference (P > 0.05) in their age, FPG, PPPG, HbA1c, serum Vitamin D, and calcium level. Age, BMI, FPG, PPPG, and HbA1c were positively connected with circulating Vitamin D and calcium, whereas age, BMI, FPG, PPPG, and HbA1c were negatively associated with those measurements. (Table 3).

The current study's findings revealed that Type 2 diabetes females had a greater shortfall in blood levels of vitamin D and calcium than Type 2 diabetic males. In terms of statistics, the difference with diabetic men was not significant (P < 0.05).

The study also revealed that normal healthy females had higher levels of vitamin D and calcium deficiencies than normal healthy men did. However, this difference was statistically insignificant [Table 5]. The current study compared the FPG, PPPG, HbA1c, and S. CAL levels of diabetic persons distributed according to S. VIT D levels and found a statistically significant difference in the levels of these variables.

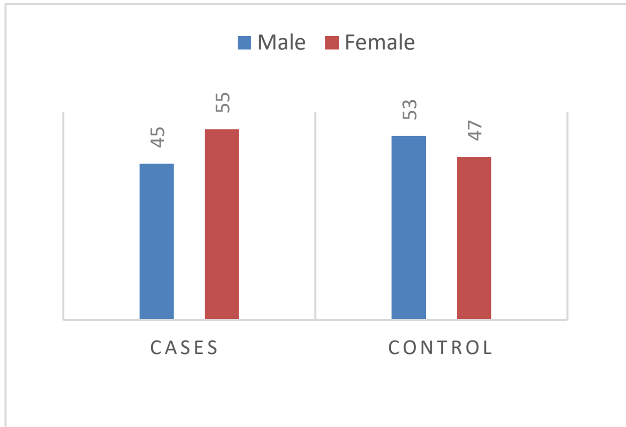

Figure 1: Gender Distribution among Case and Control groups

Table 1: Information on the demographics of Type 2 diabetes patients and healthy non-diabetic people (the "control" population)

Variable	Cases (n=100), n (%)	Controls (n=100), n (%)	χ2
Sex			
Male	45 (45.0)	53 (53.0)	0.587
Female	55 (55.0)	47 (47.0)	
Age			
30-40	17(17.0)	19(19.0)	0.488
41-50	37(37.0)	42(42.0)	
51-60	46(46.0)	39(39.0)	
Area			
Rural	56(56.0)	59(59.0)	0.715
Urban	44(44.0)	41(41.0)	
BMI			
18.5-24.9	33(33.0)	88(88.0)	0.001
25.0-29.9	43(43.0)	9(9.0)	
30.0 and above	24(24.0)	3(3.0)	

Percentages are used to display the results. P<0.05 is thought to be statistically significant.

Table2. Comparison of age, fasting plasma glucose, postprandial plasma glucose, serum Vitamin D, serum calcium Among Cases and Control groups

Variable	Cases n=100	Control n=100	P-Value
Age years	51.07±8.84	48.59±8.97	0.001
BMI (kg/m2)	27.69±2.63	22.48±2.48	0.001
FPG (mg %)	169.73±56.66	76.64±13.53	0.001
PPPG (mg%)	249.22±77.44	106.32±13.64	0.001

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HbA1c %	8.27±1.95	4.63±0.63	0.001
Serum Vitamin D (ng/ml	19.15±11.60	25.02±12.51	0.001
Serum calcium (mg/dl)	9.05±1.05	9.29±0.71	0.001

SD: Standard deviation, BMI: Body mass index; FPG: Fasting plasma glucose; PPPG: Postprandial Plasma Glucose; HbA1c: Glycated hemoglobin

Table 3: shows the relationships between fasting plasma glucose, postprandial plasma glucose, serum Vitamin D, serum calcium, and serum phosphorus in Type 2 diabetics and healthy, non-diabetic people.

Parameters	Age	BMI	FPG	PPPG	HbA1c	Serum Vitamin D	Serum calcium
Age- rho	-	0.108	0.084	0.095	0.145	−0.143	−0.065
BMI- rho	0.039	-	0.044	0.099	0.061	−0.025	−0.014
FPG- rho	0.077	0.085	-	0.754	0.782	−0.194	−0.156
PPPG- rho	0.011	0.045	0.763	-	0.645	−0.173	-0.135
HbA1c- rho	0.035	0.043	0.102	0.142	-	−0.183	-0.136
Serum Vitamin D - rho	0.056	0.045	0.005	−0.025	0.114	-	0.076
Serum calcium- rho	0.025	0.064	0.162	0.086	0.262	−0.053	-

*R: Pearson's correlation element. ** and *: The correlation is significant at a two-tailed significance threshold of 0.01 and 0.05, respectively.*

Table 4: Difference in blood levels of calcium and vitamin D among people with diabetes:

Variables	Male (n=45), n (%)	Female (n=55), n (%)	Total (n=100), n(%)	P-Value
BMI				
18.5-24.9	14(31.1)	13(26.6)	27(27.0)	0.874
25.0-29.9	22(48.8)	26(47.27)	48(48.0)	
≥30.0	9 (20.0)	16(29.9)	25(25.0)	
Serum Vitamin D				
≤30.0 (ng/ml)	31(68.88)	43(78.18)	74(74.0)	0.321
>30.0 (ng/ml)	14(31.11)	12(21.8)	26(26.0)	
Serum calcium				
<8.5 (mg/dl)	21(46.66)	31(56.36)	52(52,0)	0.214
≥8.5 (mg/dl)	24(53.33)	24(43.63)	48(48.0)	

Table 5: Difference in blood levels of calcium and vitamin D among healthy people:

Variables	Male (n=53), n (%)	Female (n=47), n (%)	Total (n=100), n(%)	P- Value
BMI				
18.5-24.9	48(90.56)	38(80.85)	86(86.0)	0.541
25.0-29.9	3(5.66)	6(12.76)	9(9.0)	
≥30.0	2(3.77)	3(6.38)	5(5.0)	
Serum Vitamin D				
≤30.0 (ng/ml)	14(26.4)	18(38,29)	32(32.0)	0.475
>30.0 (ng/ml)	39(73.5)	29(61.7)	68(68.0)	
Serum calcium				
<8.5 (mg/dl)	6(11.3)	9(19.14)	15(15.0)	0.629
≥8.5 (mg/dl)	47(88.6)	38(80.85)	85(85.0)	

Table 6: Analysis of serum calcium, glycated hemoglobin, fasting plasma glucose, postprandial plasma glucose, and serum vitamin D levels in people with diabetes.

Parameters	Categories	FPG	PPPG	HbA1c	Serum calcium
Vitamin D (ng/ml)	Severe VDD	166.80±48.41	2380.72±66.71	8.39±2.78	9.27±0.31
	Moderate VDD	148.79±38.51	216.17±38.12	7.38±2.37	9.35±0.75
	Mild VDD	133.72±26.42	185.28±37.27	7.06±1.85	8.75±1.25
	Normal	128.64±26.75	136.54±31.14	6.88±1.35	9.41±0.86
P		0.001	0.001	0.045	0.021

Discussion

The current investigation revealed that the occurrence of T2DM was higher in females (55.0%) than in males (45.0%); this conclusion was consistent with a prior study by Marwa et al., in which the percentage of T2DM in females was 63% as compared to males 37%. Additionally, the current study revealed that T2DM females were more likely to be obese (20.9%) than T2DM males (20%). This finding was in line with a prior study by McCord et al. and

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Marwa et al., which found that women are more affected by obesity than males. According to the current study, vitamin D insufficiency was more common in T2DM females (78.18%) than males (68.88%). Arif et al.'s 2017 research indicating T2DM females were four times more likely to experience vitamin D insufficiency than males (79%) and (21%), respectively, supported this conclusion.

Following a study by Bayani et al., where the 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, the vitamin D levels of patients in the current study were found to be in the range of 19.15–11.60ng/ml while those of controls were in the range of 25.02–12.51 ng/ml. The findings demonstrated that Type 2 diabetes patients had considerably lower vitamin D levels than healthy people, as shown by multiple researchers in earlier studies by Pittas et al. 2010, Anderson et al. 2010, and Knekt et al. 2008. Another major finding in line with the 2009 study by Hagenau et al. was the substantial negative correlation between age and Vitamin D levels in diabetes patients (r = 0.143). Between Type 2 diabetics and non-diabetics, there was a significant difference in blood levels of calcium and vitamin D in this research. Our findings verified research by Raab et al., Al-Shoumer et al., and Bierschenk et al. that showed T2DM patients have lower blood Vitamin D concentrations than non-diabetics. The average level of vitamin D in people with and without diabetes in this investigation indicated that both groups had a vitamin D insufficiency. However, the high frequency of vitamin D insufficiency in both groups may be partly to blame for variations in findings compared to earlier research. Adiposity, genetics, and elements that affect the dermal synthesis of vitamin D, such as season, color of the skin, melanin level, age, clothing, and intake, are some of the variables that may impact vitamin D status. According to the Gaafar and Badr et al. 2013 study findings, Type 2 diabetes patients' blood levels of 25(OH) D and calcium significantly differed from those of the control group. There seems to be a connection between calcium status in T2DM and vitamin D levels. According to this study, vitamin D deficiency affects postprandial glycemia and insulin responsiveness, and as vitamin D is required to produce insulin, it may contribute to the onset of diabetes. Since the sample size is small, more analysis using a larger sample size is necessary before drawing a conclusive conclusion.

Conclusion

Calcium and Vitamin D levels in the blood have an impact on glycemia. According to the present study's findings, vitamin D insufficiency causes calcium levels to drop, which may contribute to the development of type 2 diabetes. This study found that Type 2 diabetes patients had much lower vitamin D and calcium levels, which may play a key role in the disease's propensity to develop early in vulnerable people.

Declarations

Data Availability statement
All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate
Not applicable

Consent for publication
Approved by the Concerned Hospital

Funding
Not applicable

Conflict of interest

The authors declared an absence of conflict of interest.

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