

Original Article

The Association of Serum Calcium and Vitamin D with Insulin Resistance and Beta-Cell Dysfunction among People with Type 2 Diabetes

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Abstract

Cross-sectional studies have linked vitamin D deficiency and alteration of calcium levels to an increased incidence of type 2 diabetes and insulin resistance. This study investigated a possible correlation between blood vitamin D and calcium levels with insulin resistance and beta-cell dysfunction in type 2 diabetes, pre-diabetes, and healthy individuals. This cross-sectional study involved 300 participants. Participants were divided into three groups (n=100), type 2diabetic, prediabetic, and healthy. In order to measure insulin resistance and β -cell function, the HOMA IR and HOMA B were assessed, respectively. Also, the other parameters such as serum 25(OH)D, blood insulin (FPI), glucose (FBS), HbA1c, and calcium were assessed in this study. In simple regression analysis, a high vitamin D level is linked to lower levels of FBS, HbA1c, Insulin, and HOMA IR, and higher levels of HOMA B. Calcium has a positive connection with FBS and HbA1c and a negative connection with insulin level and HOMAB. Hypovitaminosis D may substantially influence diabetes patients' glycemic dysregulation. "An increased incidence of type 2 diabetes has been related to a disruption in calcium homeostasis. All in all an increment in calcium levels may have a role in developing type 2 diabetes".

Keywords: Type 2M, 25 (OH) D, Calcium, Vitamin D deficiency, Insulin resistance, Glycemic control

1. Introduction

Individuals, communities, and families worldwide are affected by diabetes, a chronic, life-threatening disease. It is one of the highest ten adult death causes, with an estimated 4 million people dying globally in 2017. Two of the most frequent forms of diabetes are type 1 (T1D) and type 2 (T2D) (1). Some risk factors include an increase in waist circumference, a rise in insulin levels, insulin resistance, and dyslipidemias that lead to atherosclerosis. Obesity, insulin resistance, non-alcoholic fatty liver disease, and metabolic syndrome are all linked to T2DM, cardiovascular disease, and "Fasting blood sugar (FBG) levels of 100-125 mg/dl"

are considered pre-diabetes (2). A relative "lack of insulin secretion and insulin resistance" causes type 2 diabetes (3). The major pathogenetic approaches to T2DM are chronic inflammation and disorders of insulin effect and secretion (4). Much attention has been paid to the probable non-skeletal effects of vitamin D, such as its function in pancreatic insulin secretion and action. Vitamin D deficiency has been linked to an increased risk of diabetes, impaired glucose tolerance (IGT), and decreased risk of cardiovascular disease (5). In 2012, Forouhi, Ye (6) showed that diabetes and hypovitaminosis D are connected by identifying vitamin D nuclear receptor

(VDR) in pancreatic cells. VDR polymorphism was linked to alteration of insulin secretion and sensitivity, indicating that 25-hydroxyvitamin D plays a role in both forms of diabetes etiology (7). Many intracellular insulin-mediated functions "in target tissues, including muscle and adipose tissue," require Calcium to function correctly. For effective insulin action, an optimal intracellular amount of Ca^{2+} is required. Peripheral insulin resistance can be caused by impaired insulin signaling, which is linked to lower transporter of glucose function owing to changes in target tissues' intracellular Ca^{2+} levels. Insulin sensitivity is influenced by the "1, 25(OH) $2D$, which regulates extracellular Ca^{2+} concentration and flow across cell membranes" (8). "Vitamin D deficiency has also been linked to an increase in Ca^{2+} concentration, which has been linked to a decrease in GLUT-4 function and insulin resistance. In the pancreatic β -cells, vitamin D is involved in the control of Ca^{2+} flow (9)."

This study investigated a possible correlation between blood vitamin D and calcium levels with insulin resistance and beta-cell dysfunction in type 2 diabetes, pre-diabetes, and healthy individuals.

2. Materials and Methods

2.1. Study Design

This cross-sectional study involved 300 participants and was carried out at Al-MWANEE Teaching hospital and a private clinic in Basra governorate, Iraq. Sample collection proceeds over 6 months from 1st October 2020 till first march 2021. Participants groups divided into three groups (n=100), Group 1: type 2diabetic fasting blood sugar ((FBS) \geq 126 mg/dL, HbA1c \geq 6.5%), Group 2: prediabetic (FBS (100 - 125 mg / dL), HbA1c (5.7 % - 6.4 %)), group 3: healthy subjects (FBS less than 100 mg /dL, HbA1c (4%-5.6%).

2.2. Criteria for Inclusion and Exclusion

Inclusion criteria for all groups are defined as Age range (18-70) years, gender, both male and female, BM²25- 40kg/m². Cancer, liver disease, renal disease, and severe gastrointestinal problems. Calcium and vitamin D supplement consumption. Whether the

participants are a smoker or a drinker, hypercalcemia, nephrolithiasis, previous nontraumatic fractures, being pregnant or breastfeeding, and having primary hyperparathyroidism were all ruled out.

2.3. Data Collection

2.3.1. Specimens Collection and Preparation

A questionnaire was used to gather data on "age, gender, dietary habits, marital status, medical history, lifestyle-related information, addictive behaviors, physical activity, education level, smoking status, height, weight, diabetes duration, type of diabetes medication."

After venipuncture, approximately 5ml of venous blood was collected from each fasting participant in gel vacuum collection tubes, allowed to clot at room temperature for 5-10 minutes, and then separated by centrifugation at 1000 rpm for 10 minutes to obtain serum that was kept frozen in plain tubes for analysis using automated biochemistry devices for each biomarker involving Vitamin D, Insulin, FBS, and Calcium.

About 2 – 3 ml of venous blood were collected from each fasting participant in vacuum EDTA tubes, and gentle mixing was then transferred to automated "Bio-Rad D-10" to measure "(Hemoglobin A1C) Hb A1c". Whole blood samples can be kept at "2–8 °C for 7 days or 3 days" at room temperature (15–30 °C).

2.4. Biochemical Analysis

The Bio-Rad D-10TM Hemoglobin A1c Program uses ion-exchange high-performance liquid chromatography to determine the % of hemoglobin A1c in whole human blood (HPLC). On the Cobas e 411 Analyzer, the electrochemiluminescence binding assay is intended to measure the total 25-hydroxyvitamin D in human blood and plasma. On the Cobas e 411 Analyzer, Insulin was measured using the sandwich principle. The enzymatic method with hexokinase was used to measure fasting blood sugar on Cobas Integra 400 plus biochemistry Analyzer. The calcium EDTA complex method was used to measure the serum concentration of Calcium on Cobas Integra 400 plus biochemistry Analyzer. The HOMA of insulin resistance (HOMA-IR) index is used to assess insulin

resistance, whereas the HOMA of β -cell function (HOMA-B) index is used to assess β -cell function (10).

"HOMA of insulin resistance(HOMA-IR)"

"HOMA-IR = [FBS(mg/dL)×Fasting insulin (μ IU/ml)]/405"

"HOMA of β -cell function (HOMA-B)"

"HOMA-B =[360 × Fasting insulin (μ IU/ml)]/[FBS (mg/dL) – 63]"

2.5. Statistical Analysis

The statistical data were analyzed using SPSS version 24. Numbers and percentages were utilized to convey qualitative data, while quantitative data was represented by the mean, standard deviation, median, lowest and highest values. The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to examine the distribution of quantitative data. Qualitative data were analyzed using the Chi2 test, while non-parametric data were analyzed using the Kruskal Wallis and Spearman correlation tests. The linear regression between any two associated quantitative variables was depicted using simple linear regression diagrams. Statistical significance was a

probability (p) value less than or equal to 0.05.

3. Results

The study population had the following demographic and physical characteristics:

Demographic and physical characteristics of one hundred fifty individuals divided by diabetes, pre-diabetes, and healthy persons as the control group included in this study, as shown in table 1, indicate that there was no significant difference between diabetes, pre-diabetes, and controls for the mean of age "($P>0.05$) (47.48±10.70), (42.64±10.65), (44.54±8.43)" respectively. "There was no significant difference ($P>0.05$) between groups regarding sex, and there was no significant difference concerning BMI between patients and controls ($P>0.05$)".

3.1. Biochemical Parameters

As shown in table 2, FBS and HbA1c serum levels are substantially greater in diabetes and pre-diabetes than in the control group ($P<0.001$). Similarly, serum levels of these parameters are also significantly higher in diabetes than in pre-diabetes.

Table 1. Distribution of the study population according to demographic and anthropometric characteristics

		Category			Sig.
		Control	Pre-diabetes	Diabetes	
Sex	Male	26 52.0%	24 48.0%	22 44.0%	0.73*
	Female	24 48.0%	26 52.0%	28 56.0%	
Age (year)	Mean± SD	44.54±8.43	42.64±10.65	47.48±10.70	0.061**
	Median	44.00	44.00	45.50	
	Min. - Max.	30- 67	26- 66	19- 68	
BMI (kg/m ²)	Mean± SD	32.10±3.96	31.42±4.42	30.58±4.12	0.106**
	Median	33.00	31.00	29.60	

"Chi-squaretest, **kruskal wallis test, BMI: body mass index". " P value<0.05 considered significant"

Table 2. Differences in FBS and HbA1c levels among the three categories of the study population

		Category			Sig.
		Control	Pre-diabetes	Diabetes	
FBS	Mean± SD	89.26±5.51	119.52±4.50	221.24±105.06	0.0001*
	Median	89.00	119.50	194.50	
	Min. - Max.	79-101	103-128	68-572	
A1c	Mean± SD	5.09±0.43	6.22±0.21	9.83±1.99	0.0001*
	Median	5.00	6.30	9.55	
	Min. - Max.	4.40-5.90	5.80- 6.50	7.10-14.40	

"The information is given as a mean and standard deviation" (SD),* "Kruskal Wallis test, FBS: fasting blood sugar (mg/dL)", A1c: glycated hemoglobin A1c (%)," P value <0.05 considered significant".

Table 3 "shows a substantial variation in vitamin D and calcium levels across groups. 25(OH)D concentrations in the control group exceeded those in the diabetic and prediabetic groups. The mean calcium level in the diabetes group was greater than that in the pre-diabetes and control groups, respectively".

The biochemical variables in table 4 indicate that Insulin and HOMA-B were significantly higher in pre-diabetes than diabetes and control group, while HOMA-IR was significantly higher in diabetes than in the pre-diabetes control group.

3.2. Correlations of Biochemical Parameters by Using Spearman Correlation Coefficient Test

Table 5 shows that vitamin D have a substantial negative relationship with FBS, HbA1c, HOMA-IR $R=-0.454$, $P<0.001$, $R=-0.466$, $P<0.001$, $r=-0.329$, $P<0.001$ respectively. Significant positive correlation was found between vitamin D and HOMA-B, $R=0.254$, $P<0.01$.

Significant positive correlation was found between serum calcium and FBS, $HbA1cR=0.218$, $P<0.01$, $R=0.258$, $P<0.01$ respectively. A statistically significant negative connection between serum calcium and Insulin, HOMA-B was discovered. $R=-0.185$ $P<0.05$, $R=-0.345$ $P<0.01$ respectively.

Table 3. Differences in Vitamin D and Calcium levels among the three categories of the study populations

		Category			Sig.
		Control	Pre-diabetes	Diabetes	
25 (OH)D	Mean± SD	27.44±8.91	20.99±11.71	15.10±7.26	0.0001*
	Median	28.30	19.30	15.17	
	Min. - Max.	9.30-56.30	3.90-56.80	3.02-33.46	
Ca ⁺⁺	Mean± SD	8.88±0.53	8.90±0.62	9.44±0.85	0.001*
	Median	8.80	8.70	9.47	
	Min. - Max.	8.00-10.50	7.80-10.50	7.42-10.91	

The information is provided as a mean with a standard deviation (SD), * Kruskal Wallis test, 25(OH)D: 25 hydroxy vitamin D (ng/ml);Ca⁺⁺: serum calcium (mg/dL),"(P value <0.05 considered significant)".

Table 4. Differences in HOMA-IR and HOMA-B levels among the three categories of the study populations

		Category			Sig.
		Control	Pre-diabetes	Diabetes	
Insulin	Mean± SD	7.44±4.32	17.68±8.12	24.05±27.76	0.0001*
	Median	5.55	17.90	14.62	
	Min. - Max.	3.40-19.90	6.80-33.90	2.10-135.00	
HOMA-IR	Mean± SD	1.62±0.93	5.21±2.38	13.39±18.22	0.0001*
	Median	1.23	5.37	7.35	
	Min. - Max.	0.72-4.77	1.83-9.96	0.58-111.67	
HOMA-B	Mean± SD	110.46±75.15	113.63±55.18	134.39±425.53	0.0001*
	Median	76.84	109.50	41.74	
	Min. - Max.	33.16-339.16	42.00-247.50	5.19-2952.00	

"The information is given as a mean and standard deviation (SD), * Kruskal Wallis test, Insulin; Fasting Insulin (μU/ml), HOMA-R; homeostatic model assessment of Insulin Resistance, HOMA-B; homeostatic model assessment of β-cell function.(P-value<0.05 considered significant)"

Table 5. Spearman's Correlations between different parameters in the studied population

		BMI	FBS	A1c	"25(OH)D"	Ca++	"Insulin"	HOMA-IR"	"HOMA-B"
Age (Year)	R	-.068-	.091	.150	-.051-	.190	-.072-	-.008-	-.160-
	Sig.	.407	.269	.067	.539	.020	.378	.925	.051
BMI	R		-.184-	-.129-	.156	-.065-	.115	.051	.239
	Sig.		.024	.116	.056	.431	.162	.537	.003
FBS	R			.814	-.454-	.218	.395	.702	-.436-
	Sig.			.000	.000	.007	.000	.000	.000
A1c	R				-.466-	.258	.393	.654	-.328-
	Sig.				.000	.001	.000	.000	.000
25(OH)D	R					-.130-	-.155-	-.329-	.254
	Sig.					.112	.058	.000	.002
Ca++	R						-.185-	-.025-	-.345-
	Sig.						.024	.759	.000
Insulin	R							.904	.596
	Sig.							.000	.000
HOMA-IR	R								.241
	Sig.								.003

Correlation is significant at the $P < 0.05$ level

4. Discussion

The mean BMI was "32.10±3.96, 31.42±4.42, and 30.58±4.12 kg/m²" for control, pre-diabetes, and diabetes groups, respectively, which is consistent with Pittas, Dawson-Hughes (5) study. The mean BMI of participants was "32.1 ±4.5 kg/m²," which is consistent with Niroomand, Fotouhi (11)'s study" (the mean BMI was 31±6 kg/m²). The mean age was "44.54±8.43, 42.64±10.65", and "42.64±10.65" years for the control, pre-diabetes, and diabetes groups, respectively, which is comparable to Niroomand, Fotouhi (11) study (45 ±14 years). A similar study (12) (45.8± 13.5) and lower to Branco, Smoraog (13), where the mean of age "59.47 ± 6.47 years. Another study revealed lower BMI like Alaei-Shahmiri, Khamseh (14), where the median BMI was "26.81 kg/m²". In the present study vitamin D deficiency has been linked to an increased risk of elevated FBS and HbA1C levels. Vitamin D was significantly reduced in people with "type 2 diabetes" compared to pre-diabetes and controls, and in pre-diabetes if compared with controls, as shown in table 2. This outcome was similar to other research (14-16). Low vitamin D negatively affects increasing FBS and HbA1C, as shown in tables (3-5). Other studies found the same results (17-20). Even among physically active

people, Haslacher, Nistler (21) found no link between vitamin D deficiency and hyperglycemia. Similar findings were found in systematic evaluations of vitamin D supplementation and glucose metabolism (22).

Direct binding to vitamin D receptors (VDR) in pancreatic β-cells is the method through which 1,25(OH)₂D₃ exerts its action. Pancreatic cells also contain the 1α-hydroxylase, which converts vitamin D from inactive to active form. As a result, vitamin D directly influences insulin release by inducing the vitamin D response element (VDRE) in the insulin gene's promoter region. Vitamin D regulates insulin exocytosis by raising intracellular Calcium (14, 19, 20). Table 4 demonstrates a significant association of vitamin D with HOMA IR. HOMA IR in diabetes was higher than in pre-diabetes and control. A statistically significant inverse relationship between Vitamin D and HOMA-IR is documented (5). This finding agrees with several observations demonstrating a similar relationship (9, 23, 24). In contrast, other studies revealed that HOMA IR was not associated with vitamin D (25). We found a statistically significant positive association between vitamin D and HOMA B table 5. This finding is supported by previous studies

(12, 26). However, other studies suggested that vitamin D was not associated with HOMA B (27). On the other hand, a sectional study conducted by Tao, Zhang (28) revealed a negative association between HOMA B and vitamin D.

The present study found that insulin level was lower among the control group than in the pre-diabetes and diabetes groups table 4, where vitamin D was reduced (diabetes and pre-diabetes). Similarly, Insulin and vitamin D have a negative association. But with lower significance ($R=0.155$, $P=0.058$) table 5. This finding follows several research papers (15, 29). In discordance with other studies (30), The possible explanation for these findings is that 1,25(OH)₂D binds to VDR in tissues other than pancreatic β cells like adipose tissue, muscles, and liver increasing insulin sensitivity by increasing insulin receptor expression. As a result, insulin receptor expression will be increased and, finally, proper insulin signaling (8, 26). Secondly, hyperparathyroidism secondary to Vitamin D deficiency increases insulin resistance by reducing the level of GLUT1 and GLUT4 in the cell membrane, thereby reducing glucose uptake (9, 14, 23). Low vitamin D levels in these individuals can be attributed to various factors, including less exposure to natural light, skin hyperpigmentation, and an imbalanced diet primarily confined to vegetables (12).

Furthermore, obesity is essential in decreasing 25(OH)₂D serum concentration because of the sequestration of vitamin D in adipose tissue (31). We documented a higher serum level of Calcium among the diabetes group than control and pre-groups, as shown in table 3. This finding agrees with correlation data that revealed a substantial positive relationship between serum calcium levels and indicators of hyperglycemia (FBS and HbA1C), as mentioned in table 5. These findings are consistent with several studies (32, 33). However, there are some inconsistent results (34, 35). Regression analysis demonstrates that serum calcium level has a significant inverse relationship with insulin level and β -cell function (HOMA B), as in table 5. The previous studies have the

same finding as Kim, Kim (36) for a negative association with HOMA B and Dos Santos, de Padua Cintra (37) for inverse association with insulin level. In contrast, other studies have demonstrated inconsistent results (38). At the same time, another previous report showed inconsistent findings for Association with β -cell function (10).

The possible mechanism by which serum calcium may affect glucose metabolism is by the process of insulin secretion, which is highly dependent on intracellular Calcium concentration that is affected by Calcium influx into pancreatic β -cells by voltage-gated Calcium channels. The imbalance between extracellular and intracellular Calcium of the pancreatic β -cell leads to impairment of these channels and then adversely affects the secretory function of Insulin (32).

Glycemic dysregulation in diabetic individuals may be exacerbated by "hypovitaminosis D. Type 2 diabetes" is associated with an increased risk of calcium homeostasis alteration. Type 2 diabetes may be caused by elevated amounts of Calcium in the bloodstream. It is necessary to do more research because this study did not include parathyroid hormone measurements. A bigger sample size is required for further investigation. Supplementation of vitamin D may have a role in the prospective relationship between vitamin D and disorders of type 2DM.

Authors' Contribution

Study concept and design: Z. R. H. and Q. A. Q.

Acquisition of data: Z. R. H. and Q. A. Q.

Analysis and interpretation of data: Z. R. H.

Drafting of the manuscript: Q. A. Q.

Critical revision of the manuscript for important intellectual content: Z. R. H. and M. H. A.

Statistical analysis: M. H. A.

Administrative, technical, and material support: Z. R. H., Q. A. Q. and M. H. A.

Ethics

The "Ethical Committee of the College of Pharmacy" at the University of Basra approved this investigation.

All individuals agreed to participate in the study, sample collection, and complete information; medical history was also recorded.

Conflict of Interest

The authors declare that they have no conflict of interest.

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