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Original research article

Association of insulin resistance with Vitamin-D in diabetic patients with or without microvascular disease

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Abstract

Background: T2DM and vitamin D insufficiency share risk factors such as American-African ancestry, obesity, ageing, and poor physical activity. There is also evidence linking vitamin D insufficiency to illnesses such as osteoporosis, cardiovascular disease, and metabolic syndrome problems.

Objective: This study aimed to investigate the impact of vitamin D supplementation on insulin resistance in individuals with T2DM.

Material and Method: The present study was conducted in the Department of Biochemistry. A total of 600 subjects were included in this study, which further divided into three groups (control, T2DM without microvascular complications, and T2DM with microvascular complications) SPSS software is used for statistical calculation.

Result: The difference in Vitamin D level between control and group-I is significant (P =0.044) while the difference between control and group-II is statistically significant (P=0.001). In addition, the comparison of study group-I and study group-II is statistically significant p=0.001 and mean difference is 3.69.

Conclusion: Vitamin D appears to help diabetes management, and vitamin D supplementation is advised as part of the treatment for type 2 diabetes.

Keywords: T2DM, microvascular complications and Vitamin-D

1. Introduction

Several non-skeletal disorders have been linked to vitamin D insufficiency in recent decades, including type 2 diabetes melitus (T2DM) [1].

T2DM and vitamin D insufficiency share risk factors such as American-African ancestry, obesity, ageing, and poor physical activity [2]. There is also evidence linking vitamin D insufficiency to illnesses such as osteoporosis, cardiovascular disease, and metabolic syndrome problems [3-5].

Some studies have found a link between vitamin D insufficiency and Type 2 Diabetes [6]. Other investigations have found that vitamin D may have a functional role in glucose tolerance by influencing insulin secretion and sensitivity [7].

Subjects with T2DM had considerably lower circulating 25(OH)D concentrations than healthy controls [8]. Also, vitamin D insufficiency is more frequent in women with T2DM, while elderly men with vitamin D deficiency secrete more insulin after glucose consumption [9, 10].

Animal investigations have demonstrated that vitamin D is a fundamental component required for proper insulin production [11, 12]. Vitamin D decreases insulin resistance, most likely through its influence on calcium and phosphorus metabolism and by upregulating the insulin receptor gene [13].

One research of 5,677 people with poor glucose tolerance found that vitamin D treatment improved insulin sensitivity by 54% [14]. Other investigations have demonstrated that increasing vitamin D consumption enhances insulin sensitivity [15, 16].

A research of 126 healthy individuals found a link between insulin sensitivity and 25(OH)D levels and that vitamin D insufficiency negatively impacted pancreatic β-cell function [17]. One follow-up analysis of 4,843 individuals with T2DM over 20 years found that vitamin D consumption was related with a lower prevalence of T2DM [18].

T2DM is characterised by insulin resistance and β-cell dysfunction [19]. Studies on the relationship between insulin secretion and serum vitamin-D have been conflicting. We investigated the impact of vitamin D supplementation on insulin resistance in individuals with T2DM.

2. Material and Method

Subjects and Study design: The present study was conducted in the Department of Biochemistry. The study was approved by the Ethical Committee of Institution. A written informed consent, in the vernacular language, was obtained from all the participants, upon fulfilling the inclusion criteria.

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Selection of Subjects: 600 subjects were planned to be enrolled in the present study.

Control group: Comprising 200 healthy age and sex-matched subjects selected from the staff. These subjects was non-diabetic, physically active and their fasting blood glucose level ranging from 70-100 mg %. All the patients were in the age group of 35-70 years. Both the sex groups were included. They were free from any major ailment, which could affect the parameters under study.

Test group-1: Include 200 type 2 Diabetic patients with the duration of less than 5 years. They are free from clinical evidence of any micro vascular complications of diabetes mellitus. The criteria for diagnosing diabetes will be same as laid by World Health Organization Criteria. i.e. Fasting plasma glucose ≥126 mg/dl along with classic symptoms of diabetes mellitus.
OR Postprandial plasma glucose≥200 mg/dl OR test.

Test group-2: Comprised of 200 T2DM patients suffering from one or more micro vascular complications of diabetes mellitus (either of diabetic nephropathy diabetic retinopathy or of diabetic neuropathy). Duration of the diabetes is 5 or more than five years.
To detect micro vascular complications, physician will apply the following methods.

Diabetic Retinopathy: A thorough fundus examination were done to look for retinal vascular micro aneurysms, blot and cotton wool spots (non-proliferative diabetic retinopathy) and appearance of neovascularisation (proliferative diabetic retinopathy).

Diabetic Neuropathy: A complete motor and sensory examination were carried out to any polyneuropathy, radiculopathy or mono-neuropathy. Diabetic Nephropathy: Urinary micro protein estimation along with serum creatinine will be carried out.

Exclusion criteria

- Patients with type I Diabetes Mellitus.
- Pregnant and lactating females.
- Patients taking diuretics, lipid-lowering, and multivitamins drugs.
- Patients with disease unrelated to diabetes which may alter chosen parameters i.e. AIDS, thyroid disease, tuberculosis, and cancer patients.

Sample size calculation and statistical analysis: The sample size were calculated in order to control type I & type II error. Assuming a minimum power of 80% and 95% significance level sample size will be calculated using this formula.

$$n = \frac{2(P)(1-P)\,(Z\beta + Z\alpha/2)^2}{d^2}$$

p- Incidence of the disease (Type 2 Diabetes Mellitus) q=(1-p)

$(P_1-P_2)^2$ or d^2 – Is the difference which we want to detect at a specified power & level of confidence.

Z_β - the power of statistical test we want to be minimum 80% for which is Z_β is 0.84.

$Z_{\alpha/2}$ –is the level of confidence we have chosen 95% confidence in this $Z\alpha/_2$=1.96.

When P indicates the incidence of the clinical condition e.g.: Type 2 Diabetes Mellitus.

Following the literature, the incidence of Type 2 Diabetes Mellitus will be assumed between 10%.

The calculated minimum sample size for our study is 141.

The calculated minimum sample size for the control group is 141.

In order to control loss of follow up and manual errors, for which we rounded the sample size of 200 for each group.

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Statistical Analysis: Data were collected and entered in MS Excel worksheets and results were analysed with appropriate statistical tools like student t-test, tests of significance, logistic regression analysis.

Parameters performed
1. Biochemical Parameters: Fasting Blood glucose and HbA1C.
2. Special Parameters: Serum insulin, Insulin Resistance, Serum 25Hydroxy vitamin D.

Estimation of Serum 25-Hydroxyvitamin D [20, 21]
Method: Electrochemiluminescence binding assay (ECLIA) by Cobas.
Principle: Competitive protein binding assay.
Specimen: Serum or plasma (heparin or citrate plasma) can be used in this assay.

3. Results
Total 600 subjects were enrolled in this study, which were divided in three groups. Group I consist 121 males and 79 females, group II consist 120 males and 80 females and group III consist 125 males and 75 females.

Table 1: Comparison of fasting blood glucose (mg/dl), HbA1C, Insulin and HOMA-IR levels between the groups

Groups	Description of group	Fasting blood glucose (mg/dl) (MEAN±SD)	HbA1C (MEAN±SD)	Insulin (MEAN±SD)	HOMA-IR (MEAN±SD)
Groups-I	Control	86.17±7.35	5.23±0.51	5.72±0.98	1.33±0.19
Groups-II	T2DM without microvascular complications	140±30.75*	6.87±0.75*	9.98±7.5*	3.89±2.2*
Groups-III	T2DM with microvascular complications	172.56±52.9*#	7.96±1.83*#	11.5±8.55*	5.18±2.6*

(*p<0.05 significant compared Group-I with other groups, #p<0.05 significant compared Group-II with other Groups).

Table1indicates the average fasting blood glucose (mg/dl), HbA1C, Insulin and HOMA-IR readings in three groups. Fasting blood glucose (mg/dl), HbA1C, Insulin and HOMA-IR levels increased significantly in both diabetes groups as compared to the control group. Group II exhibited a substantial difference from Group III. Group-III had the greatest rise in fasting blood glucose (mg/dl), HbA1C, Insulin and HOMA-IR compared to Groups II and I.

Table 2: Comparison of Vitamin-D3 levels between the groups

Groups	Description of group	Vitamin-D3 (Mean ± SD)
Group-I	Control	21.39±11.05
Group-II	T2DM without microvascular complications	19.19±8.21*
Group-III	T2DM with microvascular complications	15.52±7.38*,#

(*p<0.05 significant compared Group-I with other groups, #p<0.05 significant compared Group-II with other Groups)

Table-2 shows the mean Vitamin D levels in all study groups. Mean Vitamin-D level was more in control group compared other groups. The difference between Group-II and III, Iwas statistically significant. Low Vitamin-D3 was observed in Group-III.

Table 3: Comparison of mean HbA1C, HOMA-IR, vitamin-D3, MDA within the Group-II and III based on the duration of diabetes

Observation	Less than 1 year (Mean ± SD)	1-3 years (Mean ± SD)	Above 3 years (Mean ± SD)
HbA1C	6.19±0.87	7.90±1.34*	8.87±0.95*,#
HOMA-IR	1.57±0.34	3.49±0.43*	4.11±0.45*
Vitamin-D3	20.30±9.44	18.19±8.23*	16.90±7.27*,#
MDA	1.20±0.32	2.02±0.73	2.49±0.96*

(*p<0.05 significant compared less than 1 year with other time periods, #p<0.05 significant compared 1-3 years with other time periods)

HbA1C, HOMA-IR, MDA, values shown a significant increase with the duration of onset of diabetes. Highest values of HbA1C, HOMA-IR and MDA were, noticed in above 3 years duration. While Vitamin

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D3 significantly declines with the duration of diabetes.

Table 4: Comparison of mean fasting blood glucose, HbA1C, HOMA-IR, within the Group-III based on the complication

Observation	Retinopathy (n=73) (Mean ± SD)	Nephropathy (n=55) (Mean ± SD)	Neuropathy (n=23) (Mean ± SD)	Multiple complications (n=49)
FBS	151.59±44.87	187.80±52.51*	180.40±51.23*	194.42±55.04*#
HbA1C	8.16±3.77	8.40±3.23*	8.67±4.16*	9.20+5.12*#
HOMA-IR	4.39±2.94#	3.89±1.24	4.90±1.88*	4.20±1.41*#

(*p<0.05 significant compared retinopathy with others, #p<0.05 significant compared nephropathy with others)

Table-4 displaying a comparison of variables such as FBS, HbA1C and HOMA-IR within Diabetic patients, where they were further divided into Retinopathy, Nephropathy, neuropathy and multiple complications. FBS and HbA1C were significantly higher in multiple complications while HOMA-IR was significantly higher in neuropathy.

Table 5: Comparison of mean vitamin-D3, MDA, creatinine, albumin, ACR within the Group-III based on the complications

Observation	Retinopathy (n=73) (Mean ± SD)	Nephropathy (n=55) (Mean ± SD)	Neuropathy (n=23) (Mean ± SD)	Multiple complications (n=49)
Vitamin-D3	17.20±6.88	14.90±3.55	13.70±4.22	13.60±7.32
MDA	3.02±0.93	2.85±0.94	3.01±0.84	3.45±0.90

(*p<0.05 significant compared retinopathy with others, #p<0.05 significant compared nephropathy with others)

Table 5 shows the mean Vitamin D and MDA levels in group-III, which were further, divided into retinopathy, nephropathy, neuropathy and multiple complications. There was a significant increase in MDA levels in multiple complication group.

Table 6: Comparison of Vitamin D Distribution between the groups

Vitamin D levels	Control Number	%	Group-II Number	%	Group-III Number	%
Deficient (<10 ng/dl)	39	(19.5%)	35	(17.5%)	55	(27.5%)
Insufficient (10-30ng/dl)	114	(57%)	145	(73.5%)	139	(69.5%)
Sufficient (>30ng/dl)	47	(23.5%)	20	(10%)	6	(3%)

Table-6 showing the distribution of Vitamin D in all study groups where it indicating the vitamin D deficiency and insufficiency was more significant among the diabetics in compare to the normal population.

Vitamin D Levels has been analyzed between the groups. Analysis of Variance technique has been used to compare the groups. The three groups are statistically significant (P=0.001). Individual comparison between the group has been done using the Tukey HSD post HOC test and are given below.

Table 7: ANOVA for Vitamin D

	Sum of Squares	Df	Mean Square	F	p-Value
Between Groups	3505.562	2	1752.764	21.526	.001
Vitamin D within Groups	48612.765	597	81.314		
Total	52118.642	599			

Table 8: Tukey HSD

	Group	Group	Mean difference	P value
Vitamin D	Control	Diabetic without complication	2.23	.044
	Control	Diabetic complicated	5.76	.001
	uncomplicated	Diabetic complicated	3.58	.001

The difference in Vitamin D level between control and group-I is significant (P =0.044) while the difference between control and group-II is statistically significant (P=0.001). In addition, the comparison of study group-I and study group-II is statistically significant p=0.001 and mean difference is 3.69.

Table 9: ANOVA for HOMA-IR

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	Sum of Squares	Df	Mean Square	F	p-Value
Between Groups	1569.561	2	784.712	35.346	.001
HOMA-IR within Groups	13255.152	597	22.12		
Total	14824.567	599			

HOMA-IR Levels has been analyzed between the groups. Analysis of Variance technique has been used to compare the groups. The three groups are statistically significant (P=0.001). Individual comparison between the groups has been done using the Tukey HSD post HOC test and are given in Table-9.

Table 10: Tukey HSD

	Group	Group	Mean difference	P value
Homa-IR	Control	Diabetic without complication	-2.62	0.001
	Control	Diabetic complicated	-3.88	0.001
	uncomplicated	Diabetic complicated	-1.26	0.002

The difference in HOMA-IR level between control and both the study groups is statistically significant (P=0.001). In addition, the comparison of study group-I and study group-II is statistically significant p=0.002 and mean difference is -1.26.

5. Discussion

The primary goal of this study was to explore the effects of vitamin D supplementation on glucose homeostasis. The findings revealed that vitamin D administration significantly reduced blood FBG, insulin, and HOMA-IR in T2DM patients.

Vitamin D has historically been related with calcium metabolism, but in recent years, it has generated significant interest in the pathophysiology and prevention of diabetes. Some theories have been proposed to explain why vitamin D deficiency increases type 2 diabetes. Vitamin D receptors (VDRs) are expressed in adipose, pancreatic and possibly muscle cells [22, 23]. In the pancreas, vitamin D appears to modulate insulin synthesis directly through the nuclear VDR, as there are VDR elements in the insulin promoter genes [24]. Vitamin D may also promote morphological improvement in pancreatic islet cells, reduce apoptosis, and have nongenomic effects via messenger VDR [25].

In the current research investigation, we discovered that diabetic patients with microvascular difficulties were more likely to have vitamin D insufficiency than diabetic patients without microvascular issues or healthy controls. This observation was corroborated by Suzuki *et al's* investigation, as well as several previous studies [26-28].

The mean 25(OH) Vitamin D levels in both case groups were considerably lower than in controls. This points to the potential significance of 25(OH) vitamin D in the aetiology of T2DM and its consequences. This observation is consistent with those revealed by Subramanian *et al*. (2011) [26] and Yu *et al*. (2012) [29].

In our study, we tried to correlate 25(OH) vitamin D levels with FBS and HbA1C where we found a statistically significant negative correlation of 25(OH) vitamin D with FBS HbA1C.

These results are consistent with those published by Vijetha *et al*. (2014) [30] and Havilah *et al*. (2013) [31]. The negative connection between vitamin D, FBS, and HbA1c may imply that the long-term, aberrant glucose metabolism of type 2 diabetes and insulin resistance is connected with hypovitaminosis D.

In accordance to current understanding of the aetiology of T2DM, inflammation is thought to play a major role in insulin resistance, whereas cytokine-induced apoptosis may impair a-cell function. Vitamin D may protect cells from cytokine-induced apoptosis by modulating their expression.

Nevertheless, opposite results were exhibited in other research, which might be attributed to a variety of factors such as a limited number of individuals, the age of the study population, and the location of the study.

The current study found a negative association between the length of T2DM onset and mean vitamin D levels, indicating that vitamin D levels drop as diabetes progresses. Similar findings were reported by Gagnon *et al*. in 2011 [32].

6. Conclusion

Vitamin D appears to help diabetes management, and vitamin D supplementation is advised as part of the treatment for type 2 diabetes.

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