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Original Article

Investigation of the Clinical Outcomes of Long-Term Vitamin D Supplementation in Egyptian Type 2 Diabetes Mellitus Patients

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ABSTRACT

Diabetes mellitus (DM) is a group of metabolic disorders that are characterized by a chronic condition of hyperglycemia due to insulin secretion defects, insulin action, or both. The predominant form of diabetes is Type 2 Diabetes Mellitus (T2DM), which accounts for 90% of all DM cases. Studies showed that vitamin D (VD) plays an important role in changing the risk of T2DM, particularly among diabetic patients with insulin resistance. A novel association has recently been proposed between insulin resistance and vitamin D deficiency. In the current research, we investigated the association between hypovitaminosis D and T2DM. Also, we studied the effect of vitamin D supplementation on glycemic status, oxidative stress status, and inflammatory markers in T2DM patients. Forty T2DM patients with hypovitaminosis D were assessed for glycemic, inflammatory, and antioxidant parameters. After 6 months of VD supplementation for the intervention group of patients (n= 20), there was a significant improvement in VD level, Homeostatic model assessment of insulin resistance (HOMA-IR), Fasting blood glucose (FBG), glycated hemoglobin (HbAIC), serum insulin, interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), total antioxidant capacity (TAC) and malondialdehyde (MDA). In conclusion**,** as there was a significant improvement in glycemic, inflammatory, and oxidative stress parameters in type 2 diabetes mellitus patients, vitamin D supplementation has a promising effect on the treatment of type 2 diabetes.

Keywords: *Type 2 Diabetes Mellitus; cytokines; oxidative stress; Vitamin D; insulin resistance.*

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1. INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder characterized by the presence of chronic hyperglycemia accompanied by greater or lesser impairment in the metabolism of carbohydrates, lipids, and proteins. DM is probably one of the oldest diseases known to man. It was first reported in an Egyptian manuscript about 3000 years ago. In 1936, the distinction between type 1 and type 2 DM was made. Type 2 DM was first described as a component of metabolic syndrome in 1988. The origin and etiology of DM can vary greatly but always include defects in either insulin secretion or response or both at some point in the course of the disease. Type 2 DM (formerly known as non-insulin dependent DM) is the most common form of DM characterized by hyperglycemia, insulin resistance, and relative insulin deficiency. Type 2 DM results from the

interaction between genetic, environmental, and behavioral risk factors **[1].**

While the development of specific pharmacotherapy is well established and effective, there are still several limitations that include possible side effects, high cost, and long-term adherence Therefore, research has focused on discovering alternative or adjuvant therapies as an attempt to reduce the burden and costs associated with diabetes **[2].**

Vitamin D is characterized as a regulator of the homeostasis of bone and mineral metabolisms. In addition to its classical actions on mineral homeostasis, 1, 25-dihydroxy vitamin D also has nonskeletal actions. It has been reported that the brain, prostate, breast, colon, and pancreas, as well as immune cells, have vitamin D receptors and respond to this active form of vitamin D **[3]**. Vitamin D may play an important role in modifying the risk of cardiometabolic outcomes, including type 2 DM, hypertension, and cardiovascular diseases. Vitamin D deficiency (VDD) has been linked to a wide field of health problems including several types of cancer and autoimmune and metabolic diseases such as type 1 DM (T1DM) and T2DM **[4]**. A significant extra-skeletal role of VD in influencing T2DM and glycaemic control has been shown in the past decade **[5]**.

Its therapeutic potential in the treatment of insulin-resistant DM has been highlighted in recent studies. Cross-sectional studies have shown that VD modifies the risk of T2DM through modulation of pancreatic beta-cell function **[6],** sensitivity to insulin, and control of systemic inflammation **[7].** Based on these facts, short-term randomized trials in high-risk diabetic patients receiving VD supplementation compared to oral anti-diabetic therapy have recently been conducted **[8]**.

The current study aimed to find out the correlation between T2DM and hypovitaminosis D. Furthermore, the therapeutic potential of oral VD supplementation in insulin-resistant diabetic patients was also assessed by modulating glycemic status, oxidative stress, and inflammatory markers in patients with T2DM.

2. SUBJECTS AND METHODS

2.1. Subjects

It is a non-randomized non blinded study. Forty patients with T2DM were recruited for the study in Ain Shams University Specialized Hospital Diabetic Clinic. All subjects were diagnosed as T2DM and assessed for hypovitaminosis D. All hypovitaminosis D patients who were taking oral antidiabetic therapy metformin (Glucophage) are enrolled in the study they didn't take any other medications. They have a normal renal function and don't have associated comorbidities. The patients were categorized into two groups: the first group is twenty patients to be treated with VD supplement and followed-up for six months of treatment (the intervention group); while the second group is the remaining twenty patients who didn't receive VD supplement. The patients were selected according to the following inclusion criteria: established diagnosis of type 2 diabetes who take Glucophage as oral antidiabetic, VD deficiency as evidence by 25(OH) vitamin D <25 ng/mL and aged from 30 – 60 years. Elder subjects are excluded from this study. Patients with other factors that were excluded from the study included: pregnancy, lactation, hypersensitivity to VD, hypercalcemia, hypertension, and patients who received VD supplements up to 30 days before recruitment. Written consent was signed from all subjects will a full explanation of related circumstances associated with the study. The study protocol is approved by the IRB of Ain Shams University Hospitals.

2.2. METHODS

Oral liquid Vitamin D3 (Vidrop) 2800 I.U/mL was purchased from Medical Union Pharmaceuticals. Vitamin D supplements were given at a daily oral dose of 4000 IU/day for six months for the intervention group (n= 20) mixed in 100 mL orange juice. Serum and plasma samples were collected at baseline for all subjects (n= 40) and after 6 months of treatment for the intervention group (n= 20). The collected samples were used for the assessment of diabetic fasting blood glucose "FBS", Glycated hemoglobin" HbA1C", Homeostatic model assessment of insulin resistance "HOMA-IR ", plasma insulin, vitamin D (VD), tumor necrosis factor-α (TNF-α), interleukin 6 "IL-6", total antioxidant capacity "TAC", and malondialdehyde "MDA" levels.

Fasting blood glucose was measured by enzymatic colorimetric method using Glucose – Liquizyme (Single Reagent) kit (cat no 250001; spectrum diagnostics; Germany). Glycated hemoglobin was measured by enzyme-linked immunosorbent assay (ELISA) using Haemoglobin A1C (HbA1C) Human ELISA kit (cat no: E4656-100; bio vision; USA). Plasma insulin and 25(OH-vitamin D) were measured by ELISA using specific kits including Human Insulin ELISA kit (cat no: EIA2935; DRG; USA) and a 25-OH vitamin D ELISA Kit (cat no: EIA5396; DRG; USA); respectively. For inflammatory markers: IL-6 and TNF-α were measured by Human Tumor Necrosis Factor Alpha ELISA kit (cat no: EH0302; Fine Test; China) and Human IL-6 kit (cat no: ELH –IL6; Raybiotech; USA). Total antioxidant capacity and malondialdehyde were measured using colorimetric assays using Total antioxidant capacity Kit (Biodiagnostics; Egypt) and Malondialdehyde kit (Biodiagnostics; Egypt); respectively.

2.3. Statistical Analysis

The sample size was calculated using Openepi program version 3 and according to a previous study done by *Garg and Mallik (2020)* **[9]** who stated that the HbA1c was increased in the control group who did not receive vitamin D from 6.46±0.78 to 6.87±0.70 with a difference of 0.41±0.33 at 3 months follow up while the level of HbA1c was decreased from 6.40±0.65 to 5.62±0.53 with a difference of 0.78±0.27 at 3 months follow up; adjusting the power of the test to 90%; the confidence interval to 95% and the ratio between groups to 1:1; the minimum sample size needed for this study was found 28 patients divided into two equal groups each group (14 patients)

Data were collected, revised, coded, and entered into the Statistical Package for Social Science (IBM SPSS) version 23. Quantitative data were presented as mean, standard deviations, and ranges when parametric and median with inter-quartile range (IQR) when nonparametric. Qualitative variables were presented as numbers and percentages. The comparison between groups with qualitative data was done by using the Chi-square test and Fisher exact test instead of the Chi-square only when the expected count in any cell was found less than 5. The comparison between two groups with quantitative data and parametric distribution was done by using an Independent t-test while nonparametric distribution was done by using the Mann-Whitney test. The comparison between two paired groups with quantitative data and parametric distribution was done by using Paired t-test while with nonparametric distribution was done by using the Wilcoxon rank test. Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group.

3. RESULTS

3.1. Demographic and Baseline Characteristics of the studied subjects

Forty patients with T2DM and hypovitaminosis D were enrolled in the study. All patients completed the study till the end. Their mean age was 47.9±7.2, ranged from 35-59. Their mean height was 165.25±4.82. The mean weight was 82.2±13.62 and ranged from 65-114. The BMI ranged from 22- 42 with a mean value of 30.45±6.03. An overweight and obese patient with BMI (kg/m2) > 25 constitutes 75 % of the studied subjects while 25% only of subjects are of normal body weight. These results are illustrated in **table 1**.

Table 1. Baseline Characteristics of T2DM patients

		N= 40
Age (years)	Mean±SD	47.90±7.20
	Range	35–59
Height (cm)	Mean±SD	165.25±4.82
	Range	158–175
Weight (kg)	Mean±SD	82.20±13.62
	Range	65–114
BMI (kg/m^2)	Mean±SD	30.45±6.03
	Range	22–42
Period of illness (months)	Median (IQR)	49 (32.5–74.5)
	Range	3–204
Body weight status	Healthy 18–25 BMI	10 (25.0%)
	Overweight > 25 BMI	30 (75.0%)

BMI: body mass index

There was a significant difference in age and height between patients who didn't receive vitamin D and patients who received the vitamin. There was a highly significant difference in weight between the two groups with a mean weight of 71.1±4.22 in patients who didn't receive vitamin D and 93.3±10.18 in patients who receive the vitamin. About 50% of patients who didn't receive the vitamin have normal body weight with BMI (kg/m^2) <25, while 100% of those who received vitamin D are overweight and obese with BMI(kg/m^2) >25. This indicates the inverse relationship between vitamin D and BMI. These results are illustrated **in Table 2.**

3.2. Comparative analysis for baseline glycemic, antioxidant and inflammatory biomarkers in the two studied groups (patients who receive vitamin D and patients who don't receive the vitamin)

A high significant difference was observed between the vitamin D receiving group and the other group who didn't receive the vitamin for

glycemic parameters including FBG, HBA1C, plasma insulin, HOMA-IR, and 25-OH-vitamin D, Showing an increased level of FBG, plasma insulin, and HOMA-IR and HBA1c and decreased level of vitamin D in patients who receive vitamin D. Regarding the inflammatory markers (TNF-α, IL-6), there was a highly significant difference between the two groups (P= 0.001, P= 0.002) respectively, showing a higher level of inflammatory markers in those who receive the vitamin. Also, a significant difference was observed for oxidative status parameters: malondialdehyde and total antioxidants capacity (P= 0.017, P= 0.000) in the two groups respectively showing an increased level of MDA and decreased antioxidant capacity in the intervention group. Results are presented in **Table 3**.

Table 2. Comparison between patients who receive vitamin D and patients who didn't receive the vitamin regarding Demographic parameters

		Patients who didn't receive vitamin D	**Patients who receive vitamin D**	**Test value**	**P-value**	**Sig.**
		N= 20	**N= 20**			
Age (years)	Mean±SD	45.70±8.00	50.10±5.67	-2.007•	0.052	NS
	Range	35–57	40–59			
Height (cm)	Mean±SD	166.40±4.75	164.10±4.73	1.534•	0.133	NS
	Range	158–173	158–175			
Weight (kg)	Mean±SD	71.10±4.22	93.30±10.18	-9.012•	0.000	HS
	Range	65–78	80–114			
Body mass index (kg/m^2)	Mean±SD	25.50±2.01	35.40±4.36	-9.223•	0.000	HS
	Range	22–28	27–42			
Period of illness (months)	Median (IQR)	47.5 (30–77)	54.0 (36–72)	-0.488#	0.626	NS
	Range	25–100	3–204			
Body weight status	Normal body weight 18–2BMI	10 (50.0%)	0 (0.0%)	13.333*	<0.001	HS
	Overweight and obese > 25 BMI	10 (50.0%)	20 (100.0%)			

•Independent t-test; # Mann-Whitney test; *Fisher exact test

Table 3. Comparative analysis for baseline glycemic, antioxidant and inflammatory biomarkers in the two studied groups (pateints who receive vitamin D and patients who didn't receive the vitamin)

Before treatment		Pateints who didn't receive vitamin D N= 20	Pateints who receive vitamin D N= 20	Test value	P-value	Sig.
Baseline glycemic parameters						
Vitamin D (ng/mL)	Mean±SD	20.30±2.98	12.15±5.63	5.721•	0.000	HS
	Range	15–24	3.5–19			
HOMA-IR	Mean±SD	1.45±0.71	2.74±1.67	-3.178•	0.003	HS
	Range	0.7–3	1.4–7.1			
Insulin (μIU/mL)	Mean±SD	2.72±1.67	5.55±2.29	-4.461•	0.000	HS
	Range	1.2–6.5	2.4–9.3			
FBG (mg/dL)	Mean±SD	139.90±26.61	232.80 ± 78.32	-5.022•	0.000	HS
	Range	105 – 195	68.1 – 314.3			
HbA1C	Mean±SD	6.47±0.32	8.24±1.15	-6.632•	0.000	HS
	Range	6–7	6.5–10.9			
Baseline Inflammatory markers						
TNF (pg/mL)	Median (IQR)	113 (99–150)	230 (167.6–320.1)	-3.466#	0.001	HS
	Range	66–220	43.4–583.3			
IL-6 (pg/mL)	Median (IQR)	55 (40–70)	86.75 (66.4–90.5)	-3.034#	0.002	HS
	Range	15–110	18.7–168.5			
Baseline Antioxidant markers						
MDA (nmol/mL)	Median (IQR)	10.5 (5.5–15)	18.98 (7.9–22.3)	-2.384#	0.017	S
	Range	4–40	7.6 – 66.5			
TAC (mM/l)	Median (IQR)	3.95 (3.2–4.3)	2.35 (1.8–3)	4.013#	0.000	HS
	Range	2.5–5	1–3.7			

• Independent t-test; #Mann-Whitney test

3.3. Comparison between baseline and post-VD supplementation for glycemic, inflammatory, and antioxidant markers in T2DM patients who received VD supplements

There was a significant difference regarding glycemic parameters as the mean value of vitamin D is 12.15±5.63 in patients before treatment and 40.69±18.62 after treatment. Also, there was a significant difference in HOMA-IR with a range of 1.4-7.1 in patients before treatment and 0.1-1.9 in patients after treatment. Moreover, there was a highly significant difference regarding FBG and HbA1c with mean FBG 232.8±78.32 and mean HbA1c 8.24±1.15 in patients before treatment versus means FBG 113.1 ± 20.16 and mean HbA1c 6.54±0.38 in patients after treatment. Regarding the inflammatory parameters I-L6 and TNF-α, there was a significant reduction in the level of both markers after VD supplementation in patients after treatment as TNF-α range from 43.4- 583.3 and IL-6 range from 18.7-168.5 in patients before treatment versus TNF-α range from 15.4-572.2

and IL-6 range from 6.2-150.8 in patients after treatment. This emphasizes the anti-inflammatory effects of vitamin D. Finally the antioxidant effects of vitamin D appeared in a highly significant difference in MDA and TAC with range 7.6- 66.5 and 1-3.7 in patients before treatment and of the range 1.7-19.7 and 2.3-4.3 respectively in patients after treatment. These results are illustrated in **Table 4** and **Fig. 1, 2.**

Table 4. Effects of Vitamin D Supplementation on glycemic,inflammatory and antioxidant parameters in patients who receive vitamin D after six months of treatment

Pts receive vitamin D		Before treatment N= 20	After treatment N= 20	Difference Mean±SD	Test value	P-value	Sig.
Glycemic parameters							
Vitamin D (ng/mL)	Mean±SD	12.15±5.63	40.69±18.62	28.54±18.22	-7.005•	0.000	HS
	Range	3.5–19	25.2–82.2				
HOMA-IR	Mean±SD	2.74±1.67	1.24 ± 0.55	-1.50±1.52	4.414•	0.000	HS
	Range	1.4–7.1	0.1–1.9				
Insulin (μIU/mL)	Mean±SD	5.55±2.29	3.76±1.76	-1.79±1.05	7.615•	0.000	HS
	Range	2.4–9.3	0.3–6.3				
FBG (mg/dL)	Mean±SD	232.80±78.32	113.10±20.16	-119.70±84.12	6.364•	0.000	HS
	Range	68.1–314.3	89–160				
HbA1C	Mean±SD	8.24±1.15	6.54±0.38	-1.7±1.12	6.810•	0.000	HS
	Range	6.5–10.9	6.1–7.1				
Inflammatory parameters							
TNF (pg/mL)	Mean±SD	230 (167.6–320.1)	106.85 (36.5–156)	-114.36±91.07	5.616#	0.000	HS
	Range	43.4–583.3	15.4–572.2				
IL-6 (pg/mL)	Mean±SD	86.75 (66.4–90.5)	17.65 (12.6–64)	- 49.84±39.27	5.676#	0.000	HS
	Range	18.7–168.5	6.2–150.8				
Antioxidatnt parametes							
MDA (nmol/mL)	Mean±SD	18.98 (7.9–22.3)	8.35 (2.1–12.1)	-13.63±16.79	3.631#	0.002	HS
	Range	7.6–66.5	1.7–19.7				
TAC (mM/l)	Mean±SD	2.35 (1.8–3)	3.65 (3.3–4.0)	1.19±0.97	-5.496#	0.000	HS
	Range	1–3.7	2.3–4.3				

•Paired t-test; #Wilcoxon Rank test

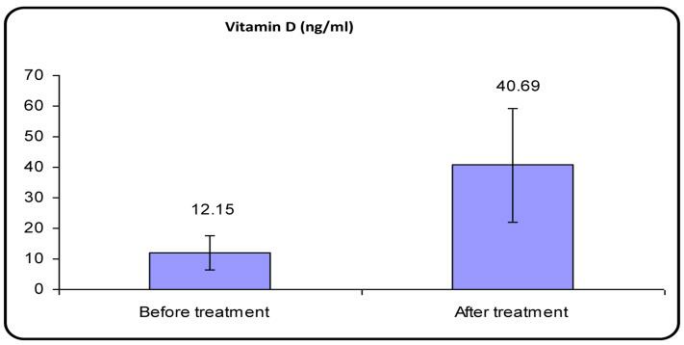

Fig. 1. Vitamin D level in patients who receive vitamin D before and after treatment

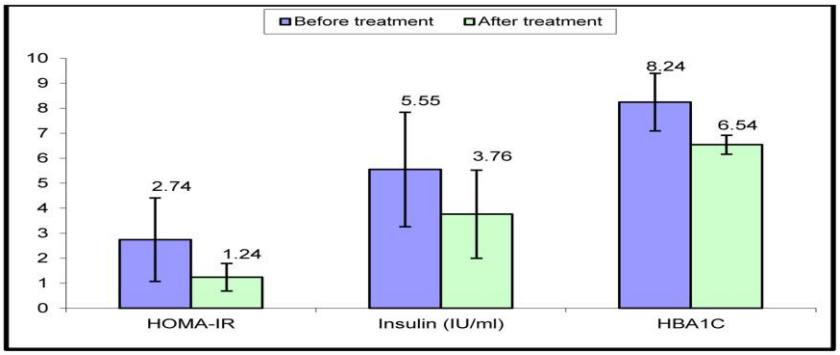

Fig. 2. HOMA- IR, insulin. HBA1C in patients who receive vitamin D before and after treatment

3.4 comparison between patients who receive vitamin D after VD3 supplementation and patients who didn't receive vitamin D3.

There was a highly significant difference in VD level and FBG between patients who receive vitamin D after treatment and patients who didn't receive the vitamin with mean Vitamin D level 40.69±18.62 and 20.30±2.98 respectively and mean FBG 113.10±20.16 and 139.90±26.61 respectively, these results are illustrated in **Table 5**.

Table 5. Comparison between patients who receive vitamin D after treatment and patients who didn't receive the vitamin regarding glycemic, inflammatory and antioxidant parameters

After treatment		patients didn't receive vitamin D N= 20	Patients receive vitamin D N= 20	Test value	P-value	Sig.
Glycemic parameters						
Vitamin D (ng/mL)	Mean±SD	20.30±2.98	40.69±18.62	-4.837•	0.000	HS
	Range	15–24	25.2–82.2			
HoMA-IR	Mean±SD	1.45±0.71	1.24±0.55	1.045•	0.303	NS
	Range	0.7–3	0.1–1.9			
Insulin (µIU/mL)	Mean±SD	2.72±1.67	3.76±1.76	-1.916•	0.063	NS
	Range	1.2–6.5	0.3–6.3			
FBG (mg/dL)	Mean±SD	139.90±26.61	113.10±20.16	3.590•	0.001	HS
	Range	105–195	89–160			
HbA1C	Mean±SD	6.47±0.32	6.54±0.38	-0.634•	0.530	NS
	Range	6–7	6.1–7.1			
Inflammatory parameters						
TNF (pg/mL)	Mean±SD	113 (99–150)	106.85 (36.5–156)	-0.300#	0.766	NS
	Range	66–220	15.4–572.2			
IL -6 (pg/mL)	Mean±SD	55 (40–70)	17.65 (12.6–64)	1.434#	0.160	NS
	Range	15–110	6.2–150.8			
Antioxidant parameters						
MDA (nmol/mL)	Mean±SD	10.5 (5.5–15)	8.35 (2.1–12.1)	1.872#	0.069	NS
	Range	4 – 40	1.7–19.7			
TAC (mM/l)	Mean±SD	3.95 (3.2–4.3)	3.65 (3.3 – 4.0)	0.675#	0.504	NS
	Range	2.5–5	2.3–4.3			

•Independent t-test; # Mann-Whitney test

Spearman correlation was made in all patients who receive vitamin D after treatment. An inverse relationship was observed between vitamin D level and HOMA-IR and serum insulin. Also, a direct relationship is observed between vitamin D level and TAC. The results are illustrated in **Table 6** and **Fig. 3, 4, 5.**

Table 6. Spearman correlation in pateints who receive vitamin D patients after treatment

	Vitamin D after treatment	
	r	**p-value**
Age (years)	-0.260	0.268
Height (cm)	-0.037	0.878
Weight (kg)	0.163	0.493
BMI (kg/m^2)	0.131	0.582
Period of illness (months)	0.147	0.537
HoMA-IR	-0.639**	0.002
Insulin (μIU/mL)	-0.474*	0.035
FBG (mg/dL)	-0.030	0.899
HbA1C	0.374	0.104
TNFα (pg/mL)	-0.043	0.859
IL-6 (pg/mL)	-0.328	0.158
MDA (nmol/mL)	-0.432	0.057
TAC (mM/l)	0.482*	0.032

Spearman correlation coefficients

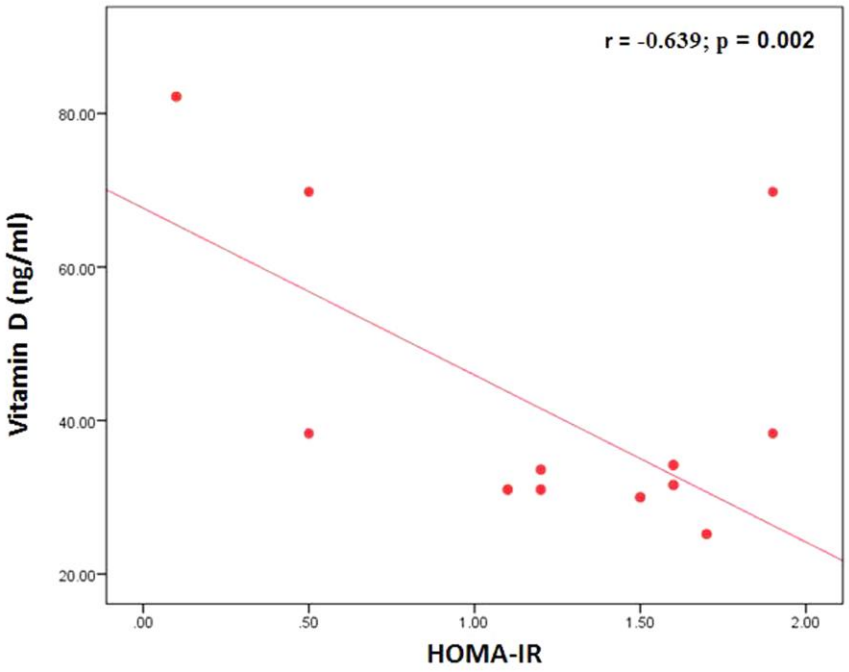

Fig.3. Spearman correlation regarding HOMA-IR in patients who receive vitamin D after treatment

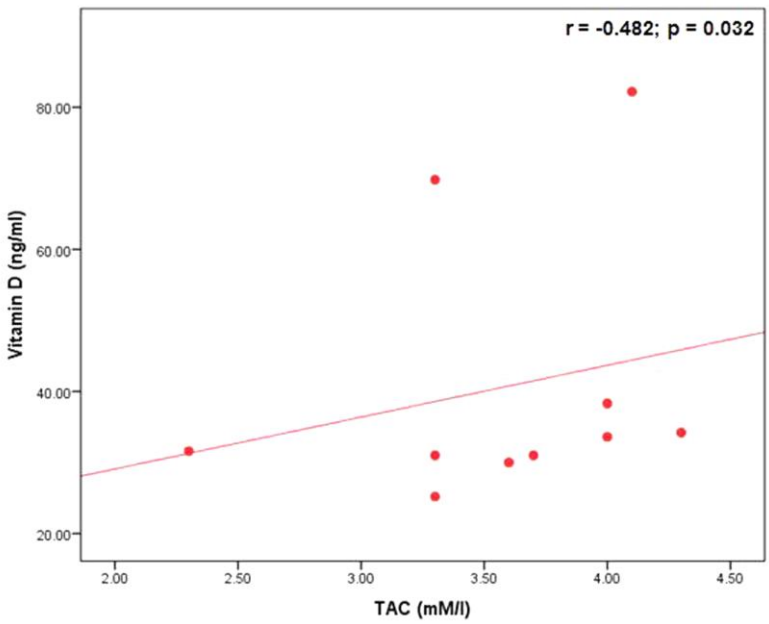

Fig.4. Spearman correlation regarding TAC in patients who receive vitamin D after treatment

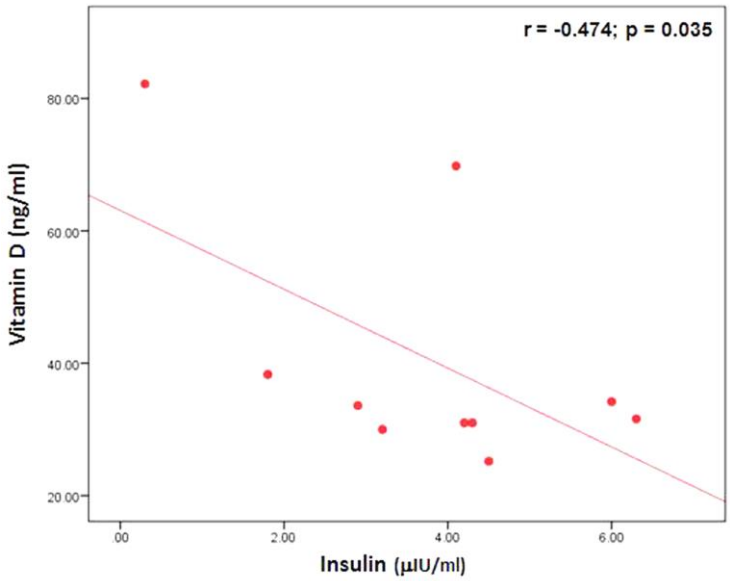

Fig.5. Spearman correlation regarding serum insulin in patients who receive vitamin D after treatment

4. DISCUSSION

DM is a group of metabolic disorders characterized by a chronic condition of hyperglycemia resulting from insulin secretion defects, insulin action, or both **[10].** It is estimated that the global prevalence of diabetes in 2019 is 9.3% (463 million individuals), rising to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045. The prevalence is higher in urban areas (10.8%) than in rural areas (7.2%) and in high-income countries (10.4%) than in

low-income countries (10.8%), (4.0%). One in two (50.1%) individuals with diabetes do not know they have diabetes **[11].** Defects in the function of pancreatic β cells, insulin sensitivity, and systemic inflammation all contribute to T2DM development. A risk factor for diabetes is insulin resistance. A novel association has recently been proposed between insulin resistance and vitamin D deficiency. VD has effects on pancreatic β-cells and insulin sensitivity in vitro and in vivo **[12].**

Our study showed that there is a strong correlation between T2DM and hypovitaminosis D between the two groups of diabetic patients (patients who receive vitamin D and patients who did not receive the vitamin) who have VDD. After VD administration, the level of VD in the intervention group also increased significantly.

This is consistent with several previous studies **[13, 14, 15, 16, 17, 18]**.

In the intervention group, our study showed a significant decrease in FBG and this is consistent with previous studies that showed that supplementation with VD resulted in a significant improvement in FBG compared to placebo in the treatment group (P= 0.03). Anyanwu *et al*. **[18]**; Bazaar *et al*. **[19]** showed that serum 25 hydroxy VD significantly reduced FBG level after 8 weeks of intervention (P= 0.04). Mirhosseini *et al*. **[8]** also indicated that in T2DM patients, VD (4000 IU/day) could significantly reduce serum FBG (P= 0.003).

Sahebi *et al.* **[20]** showed that in individuals with T2DM, VD supplementation was associated with significant improvement in FBG (P= 0.001). Our outcome, however, is contradictory to other studies that show that FBG is not significantly improved by VD supplementation **[5, 7, 21, 22].** Our study showed significant improvements in HbA1c and this is compatible with other studies that showed that HbA1C was beneficially affected by VD supplementation, which was significantly lower in the VD intervention group **[8, 15, 17]**. This result is contrary to the study by Ryu *et al.* **[16],** which showed that there was no significant difference between the placebo and VD groups in HbA1C (P= 0.415). Sadiya *et al.* **[21]** also indicated that in the VD intervention group, six months of VD supplementation had no significant difference in HbA1c. Zhou *et al.* **[23]** reported that there was a negative correlation with HbA1C in VD supplementation. In addition to the work by Al-Sofiani *et al.* **[24],** which showed that there was no significant change in HbA1C (P= 0.5) in VD supplementation for 12 weeks. It showed a significant decrease in HOMA-IR about insulin resistance, which is the main parameter in our study, and this is consistent with other studies that showed significant improvement in insulin resistance after VD administration **[17, 20].**

Bazaar *et al.* **[19]** also demonstrated that HOMA-IR was significantly reduced (P= 0.007) after 8 weeks of VD intervention. Mirhosseini *et al.* **[8]** also showed that in T2DM patients, VD (4000 IU/day) can significantly decrease the HOMA-IR index. This is contrary to other studies that show that insulin resistance is not improved by supplementation with VD **[14, 23, 25, 26, 27]**. In addition, Our study showed that before and after VD administration there is a significant change in serum insulin and this is consistent with previous studies, such as the study of Parminder *et al.* **[28],** which showed that VD supplementation significantly changes serum insulin and this is contrary to other studies which showed that replenishment with a large dose of VD did not change insulin secretion in patients with T2DM (P= 0.01) **[29].** As regards inflammatory markers (IL-6), (TNF-alpha), our study showed a significant decrease in TNF-alpha, in line with previous studies that showed that VD supplementation significantly decreases

TNF-alpha **[13, 30]**. This result, however, is contrary to the work of Yu *et al.* **[31],** which showed that supplementation with VD does not have a significant impact on TNF. Our study showed a significant decrease in IL-6 after supplementation with VD concerning IL-6, and this is consistent with several previous studies showing that VD causes a significant decrease in IL-6 **[30, 32].** This outcome contradicts the work of Yu *et al.* **[31]**, which showed that supplementation with VD does not have a significant impact on IL-6.

In addition, our study showed a significant increase in TAC and this is consistent with previous studies that showed a significant increase in TAC following VD supplementation **[33, 34]**. Our data also showed a significant decrease in MDA and this is compatible with previous studies that showed a significant decrease in MDA following VD supplementation **[33, 35]**.

In addition, our study showed that there was a significant difference in the level of vitamin D and FBG between patients receiving vitamin D after treatment and patients not receiving vitamin D.

Finally, in patients receiving vitamin D after treatment, our study showed an inverse relationship between the level of vitamin D and HOMA-IR and serum insulin, while a direct relationship between the level of vitamin D and TAC was observed in those patients.

In patients with T2DM, vitamin D has these beneficial effects because it promotes the survival of β cells by modulating the generation and effects of cytokines and nuclear factor inactivation-βB. It also acts as a depolarization modulator, stimulating insulin release through intracellular calcium regulation. In addition, vitamin D improves insulin sensitivity by stimulating insulin receptor expression and/or activating PPAR-δ, which is involved in regulating the metabolism of fatty acids in skeletal muscle and adipose tissue. As Angiotensin II inhibits the action of insulin in vascular and skeletal muscle tissue, which leads to impaired glucose uptake, vitamin D could also indirectly affect insulin resistance through RAAS. Vitamin D's anti-inflammatory effect is due to cytokine-induced Fas expression countering, as these cytokines may induce β-cell apoptosis.

Conclusion

As there is a significant improvement in glycemic, inflammatory, and oxidative stress parameters in type 2 diabetes mellitus patients; vitamin D supplementation has a promising effect on the treatment of type 2 diabetes.

Declarations

Ethics approval and consent to participate

Not applicable

Consent to publish

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article in the main manuscript.

Competing interests

No competing interests were declared by the authors.

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