Original Article

The Effect of Vitamin D Supplementation on Ankle-brachial Index in Patients with Type 2 Diabetes: A Randomized Clinical Trial

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Abstract

Background: Vitamin D deficiency in patients with type 2 diabetes (T2D) is recognized as a risk factor for developing vascular complications and poor disease control. This study aimed to determine the effect of vitamin D supplementation on ankle-brachial index (ABI) in patients with T2D. **Materials and Methods:** This two-group clinical trial was conducted among 56 patients with T2D. Participants were recruited through sampling conducted in the diabetes clinic of Velayat Hospital in Qazvin, Iran. They were allocated to the intervention and control groups using a simple blocked randomization method. The intervention group received an oral dose of 1000 IU/day of vitamin D. Data were collected using a checklist that included demographic information, laboratory measurements, and ABI. **Results:** After the intervention, the score in the intervention group (ABI < 0.9: $t_{32} = 4.082$, $p < 0.001$) was significantly different from the control group (ABI ≥ 1.3: $t_{20} = -2.711$, $p = 0.013$). After the intervention, significant differences were also observed regarding the mean ranges of vitamin D ($t_{54} = 10.07$, $p < 0.001$), fasting blood glucose (FBG) ($t_{54} = -2.97$, $p = 0.004$), 2-hour post-prandial blood glucose (2hpp) ($t_{54} = -2.55$, $p = 0.013$), and hemoglobin A1c (HbA1c) ($t_{54} = -3.02$, $p = 0.004$) between the two groups. **Conclusions:** The results of this study revealed that a daily intake of 1000 IU of vitamin D significantly improved the ABI, serum concentration of vitamin D, FBG, and HbA1c in patients with T2D. These findings suggest that vitamin D supplementation may be considered an effective approach for preventing arterial diseases and improving blood sugar control.

Keywords: *Ankle-brachial index, diabetes mellitus, peripheral arterial disease, vitamin D*

Introduction

Given its high prevalence, diabetes has become a major global health, social, and economic concern.[1] According to the statistics, the total number of people with diabetes is projected to rise to 600 million by 2035.[2] The prevalence of diabetes in Iran is reported to be about 10.3% of the whole population.[3] The disease can lead to significant complications, including microvascular and macrovascular issues, which can severely impact patients' quality of life.[4] Hence, identifying and controlling the modifiable risk factors is crucial for preventing such complications.[5] One such modifiable risk factor is vitamin D deficiency.[6] 25-hydroxyvitamin D deficiency occurs in 91% of patients with diabetes, with severe deficiency in 32% of cases.[7] Current evidence supports the role of low vitamin D levels in the pathogenesis of vascular diseases.[8] Insufficient vitamin D levels can affect the vasculature through mechanisms such as alterations in calcium-phosphate metabolism or the renin-angiotensin-aldosterone system.[9] Therefore, insufficient vitamin D levels, often resulting from sedentary lifestyles or limited sun exposure, are also commonly observed in patients with peripheral arterial disease (PAD).[10] PAD is one of the most common complications of diabetes and is diagnosed by measuring the ankle-brachial index (ABI) or ankle-brachial pressure index (ABPI).[11] This index is a simple, reliable, and non-invasive screening tool with high sensitivity and specificity. The American Heart Association (AHA) recommends using this index to screen patients with risk factors for PAD, including those with diabetes.[12]

The ABI is calculated by dividing the brachial systolic blood pressure (BSBP) by the ankle systolic blood pressure (ASBP). Ratios between 0.90 to 1.30 are considered

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normal for adults, while ratios below 0.8 indicate the presence of PAD. Lower readings (<0.7) suggest severe disease, which may lead to complications such as ulcers and gangrene.[13] Despite the availability of efficient therapeutic options, patients with PAD currently face an approximately threefold increased risk of cardiovascular mortality.[14] These complications can lead to a decrease in overall quality of life and an increased prevalence of depression among patients with occlusive PAD. Therefore, developing better and more effective treatments is crucial to reduce the risk of lower limb PAD and cardiovascular diseases in this patient population.[15,16] Given the role of vitamin D receptors in the vessel wall, it seems that insufficient levels of this micronutrient can potentially contribute to the pathogenesis of arterial diseases. Therefore, adequate vitamin D intake could potentially reduce the risk of PAD and improve disease outcomes.[17] A meta-analysis demonstrated a significant relationship between retinal vascular disorders and serum vitamin D levels.[18] In Mazidi *et al*.'s[19] study, the results showed that vitamin D can play an effective role in improving endothelial function. However, further research is needed to confirm these findings. Additionally, one study highlighted that consuming vitamin D3, as opposed to D2, may be more effective in achieving these benefits.[20]

The appropriate amount of supplementation required to improve vitamin D levels remains controversial. In the study by Andrade *et al*.,[21] it was noted that patients with chronic diseases are prescribed vitamin D supplements in different amounts (such as 200–540,000 IU), frequencies (such as daily to monthly), and forms (such as vitamin D2 or D3). Daily intake of up to 10,000 IU of vitamin D appears to be safe.[10] If further research confirms the beneficial effects of vitamin D therapy and establishes an effective dosage for diabetes control, it could become a valuable strategy for managing diabetes complications.[8] However, most previous human studies investigating the effects of vitamin D levels on diabetes complications employed a cross-sectional design, limiting the ability to draw definitive conclusions. Therefore, well-designed clinical trials are necessary to establish the clinical benefits of vitamin D supplementation in this population, including the optimal dosage regimen and duration of treatment. This study was conducted to determine the effect of vitamin D supplementation on ABPI in patients with type 2 diabetes (T2D).

Materials and Methods

This parallel randomized clinical trial (IRCT20190919044819N4) with a pre- and post-test design was conducted among 56 patients with T2D. The study was conducted in the diabetes clinic of Velayat Hospital in Qazvin, Iran. The study period spanned from February 2021 to July 2022. Patients with T2D met the inclusion criteria if they did not have any vascular intervention over the past 2 months, were willing to participate, were using oral antidiabetic medications to manage their diabetes, and were diagnosed with vitamin D deficiency (a serum concentration of 25-hydroxyvitamin D <30 ng/mL) and PAD (an ABI ratio of <0.9 or ≥1.3) during the examination by an endocrinologist. Exclusion criteria included having liver and kidney dysfunction, having any acute diseases, having a disease resulting in changes in absorption and metabolism of vitamin D and calcium (hyperparathyroidism, hypoparathyroidism, nephrolithiasis), and taking drugs affecting ossification and vitamin D metabolism (rifampin, phenytoin, and phenobarbital). Given that the main objective of this study was to investigate the effect of vitamin D supplementation on vascular complications in patients with diabetes, Cohen's d table was used to examine the required sample size. Assuming a medium effect size (r = 0.40) for a two-tailed test with a significance level of α = 0.05 (95% confidence interval) and a type II error rate of β = 0.20 (80% power), the calculated sample size was 53. To prevent the loss of samples, 10% was also added to the sample size (60 patients in total, and 30 for each group).

In this study, patients were allocated to the intervention and control groups using a simple blocked randomization method. For this purpose, blocks of four (two for each group) were prepared. The sequence within the blocks was determined based on the maximum possible states. The final allocation sequence was determined by lottery and recorded. Sealed envelopes were used to hide the allocations. The researcher who created the allocation sequence had no role in sampling. In this study, only the statistical analyst was blinded to the study. Following ethical approval from the research committee, researchers entered the study setting and recruited eligible patients. After explaining the study objectives and obtaining written informed consent, a 6-mL venous blood sample was collected from each participant. Baseline assessments included fasting blood sugar (FBG), 2-hour postprandial blood glucose (2-hPBG), glycosylated hemoglobin (HbA1c), serum 25-hydroxyvitamin D levels (D2 and D3), and high-sensitivity C-reactive protein (hs-CRP). The ABI index and demographic data were collected at baseline. These measurements were repeated 12 weeks later to obtain post-intervention values for both groups.

Intervention group: This group consisted of 28 patients. Along with receiving routine diabetes care, an oral intake of 1000 IU of vitamin D was administered to the patients, and they were asked to take one capsule daily for 12 weeks (This dosage was based on the endocrinologist's opinion and medical protocols). In addition, long-term follow-up could have better-demonstrated changes in HbA1c. Weekly follow-up of patients' conditions was performed via phone calls to control and remind them to take the capsules. It was also explained to the patients to write the time of taking the supplements in a prepared chart compiled by researchers,

and in case of side effects, write them down and inform the researchers. After the study, vitamin D supplements were also given to those with vitamin D deficiency by an endocrinologist until their vitamin D levels became normal.

Control group: This group consisted of 28 patients. After conducting laboratory tests, the results were provided to the patient (FBG, 2HPP_BG, HbA1c, and Vit D). In addition, patients in this group were explained about diabetic patient care during an educational session. Finally, the content was provided to them in the form of a pamphlet. Part of the educational content was dedicated to diet and vitamin supplements and treatment follow-up. Although the control group was not provided with 1000 IU of vitamin D, they were informed about the dosage and benefits of taking vitamin supplements. This information was provided to the control group in the form of a patient education pamphlet. The educational content emphasized dietary guidelines, vitamin supplementation (including the recommended dosage of 1000 IU of vitamin D), and the importance of treatment adherence."

For demographic information, a checklist, including age, disease duration, gender, education, marital status, economic status, type of treatment, family history, underlying diseases, history of previous diabetic foot ulcer, and smoking, was used. Laboratory measures included FBG, 2HPP_BG, HbA1c, and serum concentration of 25-hydroxyvitamin D. Serum concentration of 25-hydroxyvitamin D was detected via radioimmunoassay. It should be noted that patients' blood samples were stored at −80°C until analysis. HI-Dop vascular Doppler was performed for all participants in the supine position. For BSBP, the blood pressure cuff was wrapped around patients' upper arms, and for ASBP, it was placed on the distal end of their legs and the Doppler was placed on the posterior tibial artery. The ABI was calculated by dividing BSBP by ASBP. Ratios of <0.90 to ≥1.30 indicated the presence of PAD in both legs.[22] Collected data were analyzed by IBM SPSS Statistics version 25.0. Fisher's exact, Chi-square, and exact Chi-square tests were used to assess the homogeneity of demographic information between the two groups. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to check the hypothesis of normality of the data. Mann-Whitney and independent *t*-tests were used to examine the mean scores and ranges between the two groups. Paired *t*-tests were used to examine the differences between the mean pre- and post-test scores and ranges of the quantitative variables. A *p* value of less than or equal to 0.05 was considered significant in all tests.

Ethical considerations

This study was approved by the Ethics Committee of Qazvin University of Medical Sciences, Iran (Ethics Code: IR.QUMS.REC.1399.291). Participants provided written informed consent before inclusion. The patient consented to participate in the study. The current study was conducted according to the Declaration of Helsinki and ICH Good Clinical Practice guidelines. All patient data were anonymized and protected according to ethical standards. No complications related to the intervention were reported during the study.

Results

In this study, 56 patients with T2D participated (28 patients in each group). Of them, four patients were excluded due to unwillingness to cooperate (two in the intervention and two in the control groups). Sampling was performed within 1 year and 5 months (from February 2021 to July 2022). In terms of demographic information, no significant difference regarding age was observed between the intervention (54.57 ± 11.26 years) and control groups (57.60 ± 10.97 years) ($t_{54} = -1.01$, $p = 0.312$). In addition, no significant difference regarding disease duration was observed between the intervention (6.64 ± 6.72 years) and control groups (9.00 ± 8.55 years) ($Z = -1.31$, $p = 0.190$). Other demographic information of patients was also compared between both groups, and no significant differences were observed [Table 1]. Patients in both groups were also examined in terms of the number of pills, and no significant differences were observed according to the results of the Mann-Whitney test ($p < 0.05$). None of the patients had a history of diabetic foot ulcer, amputation, or stroke.

Before the intervention, there was no significant difference in the ABI scores between the two groups ($p > 0.05$). However, after the intervention, the intervention group's score significantly differed from the control group ($p < 0.05$). In addition, the results revealed a significant difference between the pre- and post-test scores of the ABI only in the intervention group ($p < 0.001$) [Table 2]. The difference in the ABI scores was also obtained in the pre- and post-tests. According to the *t*-test, there was a significant difference between the control and intervention groups ($p < 0.001$) [Figure 1].

Before the intervention, there were no significant differences regarding the mean ranges of FBG, 2HPP_BG, and HbA1c between the two groups, according to the results of Mann-Whitney and independent *t*-tests ($p > 0.05$). However, the results of the independent *t*-test showed that vitamin D levels in the intervention group were significantly lower than in the control group ($p = 0.010$). After the intervention, significant differences were observed between the two groups in terms of the mean ranges of vitamin D levels, FBG, 2HPP_BG, and HbA1c ($p < 0.05$) [Table 3].

In addition, the results of Wilcoxon rank-sum and paired *t*-tests revealed significant differences between the pre- and post-test ranges of vitamin D levels, FBG, 2HPP_BG, and HbA1c in both groups ($p < 0.05$) [Table 3]. Based on the *t*-test, the difference between the control and intervention groups before and after the intervention was significant [Figure 1].

Table 1: Frequency distribution of patients with diabetes according to demographic variables in three groups

Variable		Frequency (percentage)		Statistical test
		Intervention (*n*=28)	Control (*n*=28)	
Sex	Female	18 (64.30)	16 (57.10)	Chi-square$_1$=0.29, *p*=0.584
	Male	10 (35.70)	12 (42.90)	
Marital status	Married	28 (100)	27 (96.40)	*p**=0.989
	Widow	0 (0)	1 (3.60)	
Education	Under diploma	15 (53.6)	11 (39.30)	Chi-square$_2$=2.34, *p*=0.309
	Diploma	8 (28.60)	7 (25.00)	
	University	5 (17.90)	10 (35.70)	
Economic situation	Good	9 (32.10)	15 (53.60)	Chi-square$_3$=3.51, *p*=0.172
	Normal	17 (64.30)	10 (35.70)	
	Weak	2 (3.6)	3 (10.7)	
Job	Housewife	14 (50)	13 (46.40)	Chi-square$_2$=0.42, *p*=0.807
	Retire	5 (17.90)	7 (25.00)	
	Employed	9 (32.10)	8 (28.60)	
Family history	No	7 (25.00)	10 (35.70)	Chi-square$_1$=0.76, *p*=0.383
	Yes	21 (75.00)	18 (64.30)	
Underlying disease	No	16 (57.10)	14 (50.00)	Chi-square$_1$=0.28, *p*=0.592
	Yes	12 (42.90)	14 (50.00)	
Smoking	No	25 (89.30)	28 (100)	*p**=0.236
	Yes	3 (10.70)	0 (0)	

*Fisher's exact test

Table 2: Mean and standard deviation of ABI* in two groups

Variable		Mean (Std)		Test
		Intervention (*n*=20)	Control (*n*=14)	
ABI <0.9 (*n*=34)	Pre-test	0.83 (0.03)	0.85 (0.03)	t_{32}=−1.216**, *p*=0.233
	Post-test	1.00 (0.10)	0.88 (0.04)	t_{32}=4.082**, *p*<0.001*
	Test	t_{19}=−7.24***, *p*<0.001	t_{13}=−2.10***, *p*=0.055	
		Mean (Std)		Test
		Intervention (*n*=8)	Control (*n*=14)	
ABI ≥1.3 (*n*=22)	Pre-test	1.37 (0.31)	1.38 (0.03)	t_{20}=−0.773**, *p*=0.449
	Post-test	1.26 (0.44)	1.35 (0.09)	t_{20}=−2.711**, *p*=0.013*
	Test	t_7=12.21***, *p*<0.001	t_{13}=1.04***, *p*=0.315	

*Ankle Brachial Index, **Independent *t*-test, ***paired *t*-test

Table 3: Mean and standard deviation of FBG, 2HPP_BG, and HbA1c in two groups

Variable		Mean (Std)		Test
		Intervention (*n*=28)	Control (*n*=28)	
FBG***	Pre	162.67 (25.44)	160.89 (26.63)	t_{54}=0.25*, *p*=0.799
	Post	132.35 (17.24)	147.60 (20.90)	t_{54}=−2.97*, *p*=0.004
	Test	t_{27}=12.36**, *p*<0.001	t_{27}=5.86**, *p*<0.001	
2HPP_BG****	Pre	217.67 (25.44)	215.89 (26.63)	t_{54}=0.25*, *p*=0.760
	Post	180.14 (25.47)	195.89 (20.32)	t_{54}=−2.55*, *p*=0.013
	Test	t_{27}=37.56**, *p*<0.001	t_{27}=11.32**, *p*<0.001	
HbA1c*****	Pre	8.20 (0.87)	8.14 (0.93)	t_{54}=0.26*, *p*=0.822
	Post	7.38 (0.53)	7.94 (0.82)	t_{54}=−3.02*, *p*=0.004
	Test	t_{27}=10.35**, *p*<0.001	t_{27}=3.551**, *p*<0.001	
Vit D$^{\$}$	Pre	19.13 (7.04)	23.63 (5.49)	t_{54}=−2.66*, *p*=0.010
	Post	41.02 (8.28)	20.87 (6.22)	t_{54}=10.07*, *p*<0.001
	Test	t_{27}=−10.43**, *p*<0.001	t_{27}=3.34**, *p*=0.002	

*Independent *t*-test, **paired *t*-test, ***fasting blood glucose, ****two-hour postprandial blood glucose, *****glycated hemoglobin. $^{\$}$Vitamin D

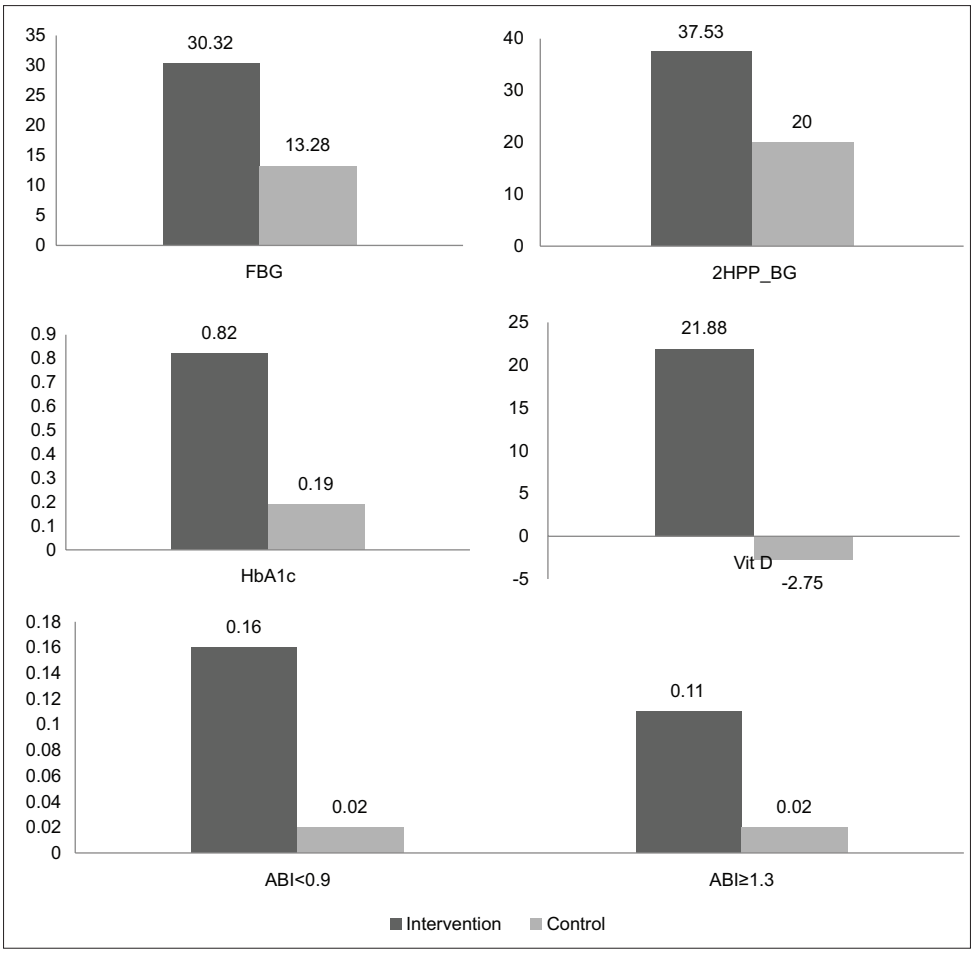

Figure 1: The mean difference between pre-test and post-test in ABI*, FBG, 2HPP_BG***, HbA1c****, and Vit D***** in two groups. *Ankle Brachial Index, **Fasting Blood Glucose, ***Two-hour Postprandial Blood Glucose, ****Glycated Hemoglobin, *****Vitamin D**

Discussion

This study aimed to investigate the effect of vitamin D supplementation on ABI in patients with diabetes. The results showed that supplementation with 25-hydroxyvitamin D significantly improved ABI. Given the high prevalence of arterial injuries and diabetic foot ulcers in patients with diabetes, it is hypothesized that vitamin supplementation may play a role in improving arterial wall health.

ABI is a simple, reliable, and non-invasive screening tool commonly used to assess PAD.[23] Except for the current study, limited studies have investigated this issue through an interventional approach. However, in one study among patients with diabetes, Depczynski *et al*.[24] showed that a high ratio of the ABI (>1.3) is associated with low serum concentration of 25-hydroxyvitamin D. In another study, it was reported that low vitamin D levels in patients with diabetes are significantly associated with the occurrence of lower extremity arterial disease.[25] In one clinical trial, Anandabaskar *et al*.[26] evaluated the effect of vitamin D supplementation on changes in vascular functions and oxidative stress among patients with T2D. Similar to our findings, their results showed that vitamin D supplementation significantly improves vascular functions and reduces oxidative stress. In their study, vascular function parameters, including carotid-femoral pulse wave velocity, brachial-ankle pulse wave velocity, arterial stiffness index, aortic pressure, and augmentation index, were measured using a periscope. Overall, the findings suggest that vitamin D supplementation may effectively improve the ABI and mitigate the progression of arterial diseases in patients with diabetes. This may contribute to the prevention of both short-term and long-term complications of the disease. Therefore, vitamin D supplementation can be considered as a potential strategy for preventing vascular diseases and their associated complications in individuals with diabetes.

As expected, vitamin D supplementation increased the vitamin D levels in the blood. Vitamin D deficiency is a common disorder in patients with diabetes and may be a risk factor for ischemic heart disease and exacerbation of diabetic nephropathy. Therefore, vitamin D supplementation is of great importance in patients with diabetes. In Momeni *et al*.'s[27] study (2017), the consumption of 50,000 IU/week of vitamin D for 2 months led to

an increase in vitamin D levels by 52 nmol/L in the intervention group. In another study, taking 5,000 IU/day of vitamin D3 for 6 months increased the serum concentration of 25-hydroxyvitamin D by 79.1 nmol/L.[28] The relationship between vitamin D supplementation and the improvement of serum concentration of vitamin D[29] was demonstrated in previous studies, and this finding is in line with our study. Therefore, physicians should be aware of the appropriate vitamin D levels for patients with diabetes to establish clear treatment goals. In addition to medication, recommendations regarding sun exposure and a vitamin D-rich diet should be provided, taking into account cultural and social factors.

In this study, laboratory measurements, including FBG, 2HPP_BG, and HbA1c, were also investigated in addition to the ABI. The results showed that vitamin D significantly reduces FBG and HbA1c. The results of Safarpour *et al*.[30] (2018) were in line with this finding. In their study, it was found that consuming 50,000 IU/week of vitamin D for 2 months decreases HbA1c levels by 1%. Additionally, a systematic review highlighted that vitamin D supplementation may be useful for reducing FPG and HbA1c in patients with T2D who have vitamin D deficiency.[31] Other studies have also confirmed the role of vitamin D supplementation in reducing HbA1c and FBG. Vitamin D supplementation as well as higher vitamin D levels improve insulin sensitivity and are thus essential for adequate release and secretion of insulin in response to glucose.[32] Therefore, incorporating vitamin D supplementation into the treatment regimen alongside antidiabetic medications may be beneficial as part of a comprehensive diabetes prevention strategy for T2D. Healthcare providers should consider this approach when developing diabetes prevention programs.

In the study, sampling was conducted from one diabetes clinic. Patient follow-up to ensure adherence to vitamin D supplementation was carried out via phone calls. However, some patients may not have accurately reported their failure to take vitamin D supplements. To address this, weekly reminders were provided by the research team. Additionally, patients in the control group might have used supplements or referred to another physician due to the low vitamin levels, which could affect the generalizability of data. As much as possible, weekly follow-ups were conducted to gather information about physician visits and use of other medications. If necessary, such patients were excluded from the study.

Conclusion

The results of this study demonstrated that daily supplementation with 1000 IU of vitamin D significantly improved the ABI, serum concentration of vitamin D, FBG, 2HPP_BG, and HbA1c levels in patients with T2D. These findings suggest that vitamin D supplementation may play a role in preventing arterial diseases and improving glycemic control. Additionally, the use of vitamin D alongside antidiabetic drugs could facilitate better blood sugar control. However, to generalize the findings of this study, further research is needed to determine the optimal dosage of vitamin D for patients with diabetes.

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Conflicts of interest

Nothing to declare.

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