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Original article

Effectiveness of vitamin D3 in facilitating the therapeutic progression of healing diabetic foot ulcers

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Abstract

Background: Diabetes and its complications afflict a large portion of the population worldwide, and it is estimated that its prevalence in all age groups will increase from 2.8% in 2000 to 4.4% in 2030. Moreover, it is well known that vitamin D may cause rapid wound healing and exert anti-inflammatory effects.
Objective: This study aimed to investigate the efficacy of vitamin D3 on the healing of chronic diabetic foot ulcers to improve the quality of life of patients with diabetes. The present research is conducted as a randomized controlled trial.
Methods: Forty patients were randomly divided into an experimental group (vitamin D supplementation) and a control group (placebo) using a sampling method based on the inclusion criteria. The patients were evaluated for ulcer debridement every two weeks (0 (T0), 2 (T1), 4 (T2), 6 (T3), 8 (T4), 10 (T5), and 12 (T6)) after the intervention. The collected data were analyzed using SPSS-23 software.
Results: The experimental group exhibited significantly decreased ulcer length (-1.6 ± 0.8 cm *vs.* -0.5 ± 1.4 cm, $P = 0.005$), ulcer width (-1.7 ± 1.2 cm *vs.* -0.9 ± 1.2 cm, $P = 0.025$), and ulcer depth (-0.6 ± 0.5 cm *vs.* -0.3 ± 0.4 cm, $P = 0.025$) after 12 weeks of intervention compared to those of the control group. These results reveal that vitamin D had a significant relationship with all stages of the wound-healing process ($P < 0.01$).
Conclusions: In patients with chronic diabetic foot ulcers, the use of vitamin D supplementation can improve wound healing. Moreover, vitamin D had a significant relationship with all stages of the wound-healing process except re-epithelialization and angiogenesis.

Keywords: Metabolic status, vitamin D, vitamin D supplementation, wound healing.

Diabetes and its complications affect a large portion of the world's population, and it is estimated that its prevalence in all age groups will increase from 2.8% in 2000 to 4.4% in 2030.[1] Despite recent medical and surgical advances, challenges related to diabetic foot complications remain a health problem and include the single biggest risk factor of non-traumatic foot amputations.[2] The probability of a patient with diabetes suffering from foot lesions, such as ulcers and gangrene, throughout their life is estimated to be 1.4%, and more than 15.0% of these wounds will eventually result in limb amputation.[3, 4] The risk of developing a diabetic foot ulcer for the first time increases in patients with a history of diabetes for more than 10 years, those who are male, those with poor blood sugar control, and those suffering from cardiovascular, kidney, and/or eye diseases.[5–7] Diabetic foot ulcers are a complex and chronic disease that has a long-term effect on a person's discomfort, mortality, and quality of life.[8] In developed countries, 15.0%–25.0% of health resources are allocated to the treatment of diabetic foot ulcers.[7, 9]

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Vitamin D deficiency is very common in elderly patients who suffer from diabetes.[10] According to previous studies, low serum levels of vitamin D are associated with a higher prevalence of diabetic foot ulcers in elderly patients.[11–13] Mean serum levels of 25-hydroxyvitamin D, or vitamin D3, steadily decrease as the severity of diabetic foot ulcers increases.[14] To prevent and control diabetic foot ulcers, elderly people who suffer from diabetes should undergo regular vitamin D screening or receive vitamin D supplements.[15] The evidence suggests that vitamin D supplements exhibit favorable effects on wound healing, inflammation biomarkers, oxidative stress, and insulin resistance, which may be due to their effect on killing bacteria by macrophages and stimulating phagocytosis.[16]

Considering the findings of previous studies and the available evidence, it is likely that vitamin D may cause rapid wound healing. This study was performed to evaluate the efficacy of vitamin D3 supplementation on the healing of chronic diabetic foot ulcers to improve the quality of life of patients with diabetes.

Materials and methods

This study was approved by the Ethics Committee of Amir al-Momenin Hospital (IRB no. 0205) and conducted in accordance with the Declaration of Helsinki.

This study is a randomized controlled trial that was conducted in Amir-Al-Momenin Hospital. Written informed consent was obtained from all patients at the beginning of the study. Sampling was performed using the readily available method, i.e., convenience sampling, and the selected patients were then divided into two groups using a simple randomization method. Each group consisted of 20 patients, where the experimental group received vitamin D supplementation and the control group received a placebo. Thus, a total of 40 patients were recruited between January 2023 and June 2023.

Inclusion and exclusion criteria

All patients aged 40–90 years with grade 3 diabetic foot ulcers were included in the study. Pregnant and breastfeeding patients with diabetic foot ulcers, patients with a history of taking vitamin D supplements in the last three months, and patients with a history of chronic trauma were excluded from the study.

Interventions in the experimental and placebo groups

All patients were randomly classified into two groups to take 50,000 IU vitamin D3 supplements (n = 20) or a placebo (n = 20) every 2 weeks for 12 weeks. Placebo capsules were similar in shape and size to the vitamin D3 supplements. All patients were evaluated for ulcer debridement every two weeks (0 (T0), 2 (T1), 4 (T2), 6 (T3), 8 (T4), 10 (T5), and 12 (T6) after the start of intervention.

Data collection tools

Wound measurements were performed by multiplying the largest diameter by the sum of the two perpendicular diameters, the second perpendicular diameter of the skin lesion, and the wound depth (cumulative wound size) was calculated for each patient. Patients were considered positive for infection if they had at least two of the following characteristics: edema, erythema, discharge, regional lymph node enlargement, pain, or fever. Each leg was evaluated and graded according to Wagner's classification.[17]

Biochemical assessment

Fasting blood samples (10 mL) were gathered after 12 weeks of intervention, and the serum was separated using a centrifuge. All samples were stored at–80°C before analysis. A commercial ELISA kit (IDS, Boldon, UK) was used to determine the inter- and intra-assay coefficient variance of the serum concentration of 25-hydroxyvitamin D, which were 4.4% and 6.7%, respectively. An ELISA kit (DiaMetra, Milano, Italy) was used to determine the serum insulin concentration. Using exchange chromatography, the level of hemoglobin $_{A1C}$ (Hb_{A1C}) was measured. Enzymatic kits (Pars Azmun, Tehran, Iran) were used to quantify the concentration of fasting plasma glucose (FPG), serum triglycerides, and very-low-density lipoprotein (VLDL), total, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol.

Statistical analysis

For data analysis, IBM SPSS Statistics 23 software was used with a significance level of $P < 0.05$. In the inferential statistics section, variance with repeated measurement, independent *t*-test, and paired *t*- test were used. Data were represented as the mean ± standard deviation (SD). In this study, multivariate logistic regression analysis was employed as a statistical method to model the relationship between

dependent and independent variables, thereby enabling the identification of factors that may influence the wound-healing process. Thus, this method is utilized to investigate the impact of vitamin D on the wound-healing process, except for re-epithelialization and angiogenesis.

Results

Figure 1 shows the vitamin D levels in the investigated time period in the experimental and control groups.

As shown in **Table 1**, the mean age of the patients participating in the study was 58.7 ± 16.3 years in the experimental group and 62.8 ± 12.2 years in the control group; the two groups were similar in terms of age (P = 0.37). In the experimental and control groups, 45% and 20% of the patients were female, and 55.0% and 20.0% of the patients were male, respectively (P = 0.088). Both groups were similar in terms of body mass index and metabolic equivalents ($P > 0.05$). After 12 weeks of intervention, the experimental

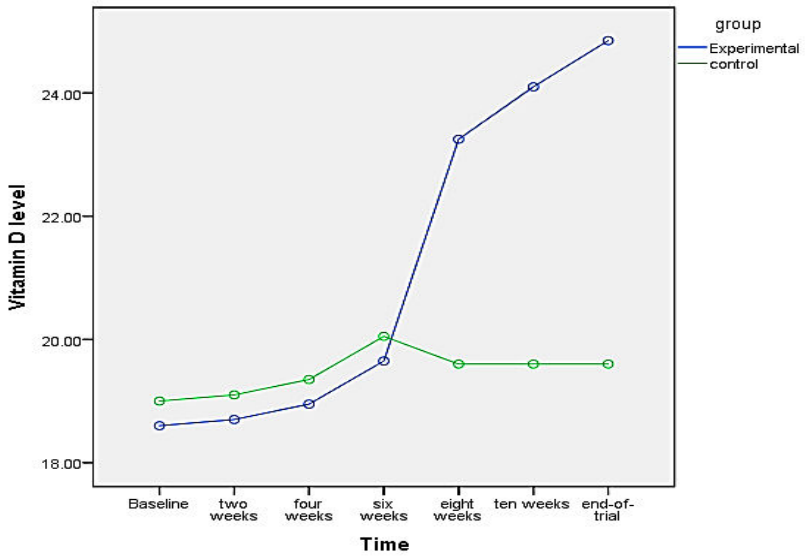

Figure 1. Vitamin D level in the investigated time period in two experimental and control groups

Table 1. Demographic and clinical characteristics of patients

Variables	Experimental group n = 20	Control group n = 20	*P*-value
Age (mean ± SD) (year)	58.7 ± 16.3	62.8 ± 12.2	0.373
Sex			
Female	9 (45.0%)	4 (20.0%)	0.088
Male	11 (55.0%)	16 (80.0%)	
Height (cm)	169.3 ± 8.8	173.4 ± 9.7	0.167
Weight at T0 (baseline)	78.0 ± 8.7	78.9 ± 6.5	0.730
Weight at T6 (end-of-trial)	77.3 ± 9.1	78.5±6.5	0.623
Weight change	0.8 ± 1.3	0.4 ± 0.5	0.195
BMI at T0 (baseline)	27.2 ± 2.5	26.4 ± 3.4	0.398
BMI at T6 (end-of-trial)	27.0 ± 2.6	26.3 ± 3.7	0.504
BMI change	0.3 ± 0.5	0.1 ± 0.2	0.169
Metabolic equivalents at T0 (baseline)	25.9 ± 1.0	25.5 ± 0.9	0.199
Metabolic equivalents at T6 (end-of-trial)	25.7 ± 1.0	25.3 ± 1.1	0.23
Metabolic equivalents change	0.2 ± 0.4	0.2 ± 0.5	1.00

group exhibited significantly decreased ulcer length (−1.6 ± 0.8 cm *vs.* −0.5 ± 1.4 cm, $P = 0.005$) **(Figure 2A),** ulcer width (−1.7 ± 1.2 cm *vs.* –0.9 ± 1.2 cm, $P = 0.025$) **(Figure 2B),** and ulcer depth (−0.6 ± 0.5 cm *vs.* −0.2 ± 0.4 cm, $P = 0.025$) **(Figure 2C)** compared with those of the control group.

In the experimental group, a change in serum insulin concentration and Hb_{A1c} was observed ($P < 0.05$) **(Table 2)** compared to those of the control group. Moreover, when comparing T0 and T6, all of the examined items were significantly altered ($P < 0.05$) **(Table 2).** The results of the multivariate logistic regression test revealed that vitamin D had a significant relationship with all stages of the wound-healing process except re-epithelialization and angiogenesis ($P < 0.01$) **(Table 3). Figure 3** summarizes the biological mechanisms by which vitamin D contributes to wound healing in diabetic ulcers.

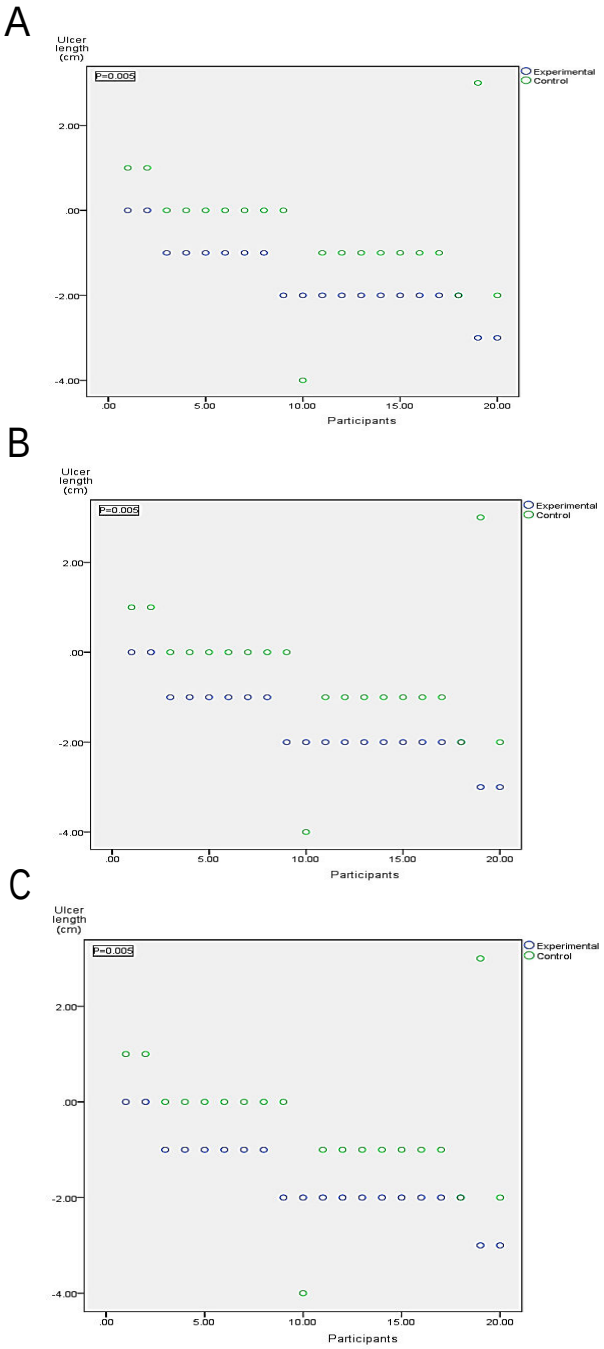

Figure 2. Scatter-gram of mean changes in **(A)** ulcer length of end-of-trial; **(B)** scatter-gram of mean changes in ulcer width of end-of-trial; and **(C)** scatter-gram of mean changes in ulcer depth of end-of-trial.

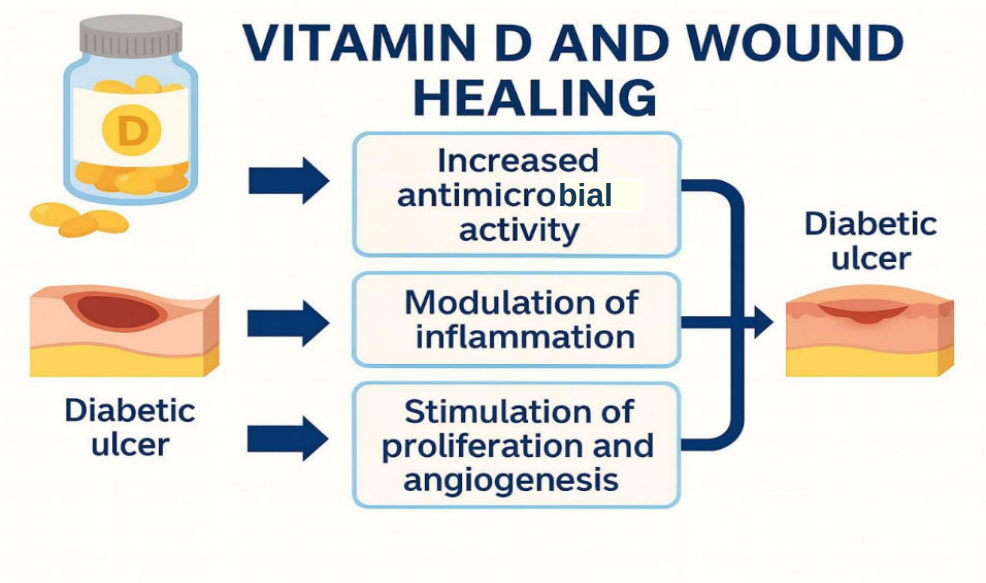

Figure 3. The biological mechanisms through which vitamin D contributes to wound healing in diabetic ulcers: enhancing antimicrobial activity, modulating inflammation, and stimulating proliferation and angiogenesis.

Table 2. Wound healing, metabolic profiles at the T0 and T6

Variables	Experimental group n = 20		Control group n = 20		*P*-value independent *t*-test		*P*-value paired-samples *t*-test	
	T0	T6	T0	T6	Before	After	Experimental	Control
Vitamin D	18.6 ± 3.4	24.9 ± 2.7	19.0 ± 3.3	19.6 ± 3.4	0.710	< 0.01	0.04	0.849
Ulcer symptoms								
Erythema (%)	20 (100)	6 (100)	20 (100)	0 (100)	0.163	0.129	0.01	0.811
Discharge (%)	20 (100)	4 (100)	20 (100)	1 (100)	0.261	0.146	0.00	0.811
Necrosis	9 (100)	2 (10)	9 (100)	0 (10)	0.367	0.273	0.02	0.811
Fasting plasma glucose	154.0 ± 25.7	139.3 ± 27.2	149.9 ± 25.1	145.1 ± 24.2	0.614	0.481	0.01	0.516
Insulin	16.2 ± 1.4	12.9 ± 1.3	16.3 ± 1.4	14.4 ± 2.3	0.745	0.012	0.03	0.784
Hb_{A1c} (%)	7.8 ± 0.7	8.1 ± 0.7	6.8 ± 0.7	7.9 ± 0.9	0.114	< 0.001	0.06	0.230

Table 3. Correlation between normal wound-healing process and vitamin D.

Phase		β	Wald	Exp (B)	*P*-value
Hemostasis	Vascular constriction	4.5	205.9	98.2	< 0.001
	Platelet aggregation, degranulation, and fibrin formation (thrombus)	2.1	51.5	10.1	< 0.001
Inflammation	Neutrophil infiltration	3.8	76.1	23.9	0.002
	Monocyte infiltration and differentiation to macrophage	5.9	58.4	33.3	0.031
	Lymphocyte infiltration	3.3	32.3	28.1	0.023
Proliferation	Re-epithelialization	0.7	81.1	0.8	0.41
	Angiogenesis	0.5	9.7	0.8	0.86
	Collagen synthesis	0.4	64.3	0.8	0.01
	ECM formation	0.1	10.1	0.8	0.89
Remodeling	Collagen remodeling	1.1	5.3	0.8	0.09
	Vascular maturation and regression	3.2	34.1	41.6	0.01

Discussion

In this study, the effects of vitamin D supplementation on wound healing in patients with chronic diabetic foot ulcers were investigated. Our findings revealed that after 12 weeks of intervention, vitamin D supplementation in the experimental group exerted significantly positive effects on the wound-healing parameters compared to those of the placebo group. In the present study, a significant decrease in serum insulin levels was observed after 12 weeks of vitamin D supplementation in the experimental group compared to that of the placebo group. The results of the present study are consistent with those previously reported, where a supplement of 50,000 IU of vitamin D was administered every two weeks for 12 weeks.[18] However, one study showed that supplementing with 1,000 IU of vitamin D in healthy overweight or obese women for 12 weeks did not affect insulin resistance.[19] These findings align with the results of Ebadi SA, *et al.*[20], who showed that in healthy overweight and obese adults, after eight weeks, the mean level of vitamin D was significantly lower in the placebo group than in the vitamin D group (administered 50,000 IU/week). The patients who received vitamin D had significantly lower levels of fasting blood sugar and fasting insulin levels than those of the placebo group.[20] Aliashrafi S, *et al*. reported similar results after 12 weeks of intervention (50,000 IU vitamin D3), with primary outcomes of changes in fasting serum glucose, insulin resistance homeostasis assessments, and matrix metalloproteinases.[21, 22]

In adult humans, optimal wound healing involves the following events: 1) rapid hemostasis; 2) appropriate inflammation; 3) mesenchymal cell differentiation, proliferation, and migration to the wound site; 4) appropriate angiogenesis; 5) re-epithelialization prompting (re-growth of epithelial tissue over the wound surface); and 6) proper synthesis, cross-linking, and alignment of collagen to provide strength to the healing tissue.[23-25] In this study, it can be concluded that there was a significant relationship between vitamin D and the normal wound-healing process.

Vitamin D contributes to wound healing through several mechanisms, as shown in this study. It plays a vital role in modulating the immune response by promoting the expression of anti-inflammatory cytokines while suppressing pro-inflammatory cytokines, thereby controlling excessive inflammation in the wound area. This immunomodulatory effect is supported by numerous studies, which showed that adequate vitamin D levels are associated with improved immune regulation and reduced chronic inflammation and are critical for optimal wound healing. In addition, vitamin D supports the proliferation and migration of keratinocytes and fibroblasts, two essential cell types involved in skin regeneration and tissue repair. This effect is consistent with previous research, which indicated that vitamin D enhances the cellular activities necessary for wound closure and tissue remodeling. Furthermore, vitamin D regulates the expression of antimicrobial peptides, such as cathelicidin, which protect against wound infections, a major concern in chronic diabetic ulcers. This antimicrobial property aligns with previous studies that emphasized the importance of innate immunity in healing processes. Moreover, vitamin D influences angiogenesis by modulating the vascular endothelial growth factor levels, thereby supporting the formation of new blood vessels essential for nutrient delivery and tissue regeneration. Adequate vascularization is a critical factor in accelerating wound healing, particularly in patients with compromised conditions such as diabetes.

Furthermore, it contributes to proper collagen deposition and extracellular matrix organization, which enhances the repaired tissue's tensile strength and structural integrity. These combined biological effects may explain the significant improvements observed in the vitamin D-treated group in this study. Similar to our findings, numerous other studies support the positive role of vitamin D in wound healing, particularly in cases of vitamin D deficiency. For instance, clinical trials have shown that vitamin D supplementation can improve wound-healing outcomes, particularly in chronic wounds such as diabetic foot ulcers. However, some studies report variable results depending on factors such as dosage, treatment duration, and patient baseline vitamin D levels, thus indicating the need for standardized protocols. The multifaceted mechanisms by which vitamin D enhances wound repair highlight its potential as a therapeutic adjunct. Moreover, addressing vitamin D deficiency in at-risk populations could be a cost-effective strategy to improve healing outcomes and reduce complications associated with chronic wounds.[23-30]

The present study had some limitations. First, the sample size was small, and it is necessary to perform

more studies with a larger sample size to confirm the current findings. There was also a significant improvement in the patients' Hb_{A1c} levels in the vitamin D group, which makes it challenging to exclude the potential glucose-lowering effect of vitamin D supplementation on wound healing, considering the baseline Hb_{A1c} levels. In the present study, it was not investigated whether the patients used insulin or not, and this variable should be addressed in future studies.

Conclusion

In the current study, the intervention was performed for 12 weeks, and the findings showed that in patients with chronic diabetic foot ulcers, the use of vitamin D supplementation improved wound healing. This result may be because vitamin D improves blood sugar control. Furthermore, vitamin D had a significant relationship with all stages of the wound-healing process except re-epithelialization and angiogenesis.

Acknowledgments
Not applicable

Conflict of interest statement
The authors have no conflicts of interest to declare.

Data sharing statement
All data generated or analyzed in the present study are included in this published article. Further details are available for non-commercial purposes from the corresponding author upon reasonable request.

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