Vitamin D supplementation improves foot ulcers among diabetic patients: Pooled analysis of randomized controlled trials

Muhammad IA. Putra1,2*, Naufal Gusti3, Teuku F. Duta4, Meulu Alina4, Intan Qanita4, Muhammad A. Naufal4, Najlaika Henira4, Ghina Tsurayya4 and Shakira Amirah5

1Department of Surgery, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia; 2Department of Surgery, Dr. Zainoel Abidin Hospital, Banda Aceh, Indonesia; 3Faculty of Public Health, Universitas Muhammadiyah Aceh, Banda Aceh, Indonesia; 4Medical Research Unit, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia; 5Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

*Corresponding author: ikhlas21@mhs.usk.ac.id

Abstract

Serum vitamin D level is associated with the development of diabetic foot ulcer (DFU), and it is intriguing to determine if the vitamin supplementation could reverse the diabetic complication. The aim of this study was to investigate the efficacy of vitamin D supplementation in DFU management through qualitative and quantitative systematic review. A systematic search was conducted to screen the records identified in PubMed, Scopus, Embase, Scillit, Sci-Finder, LILACS, EuropePMC, medRxiv, bioRxiv, Google Scholar, Semantic Scholar, and Garuda databases as of 10 March 2023. Studies investigating the efficacy of a single dose supplementation of vitamin D in DFU management were included. Quality of the included studies was appraised by Cochrane ‘Risk of Bias’ 2.0. Random-effects-based pooled analysis using Cohen’s d was performed on the wound healing progress as the primary outcome. A sensitivity test with leave-one-out method and meta-regression were also conducted to analyze the effect of heterogenous data. Five studies with a total of 245 patients (123 versus 122 for experimental and control groups, respectively) were finally included in the qualitative and quantitative analysis. The pooled estimate suggested that administering vitamin D to DFU patients could reduce the wound area or depth significantly as compared to control group (p<0.001; Cohen’s d: 2.72; 95% CI: 1.02 to 4.42). The value remained positive throughout the leave-one-out analysis. Vitamin D supplementation significantly contributed to the increased level of serum vitamin D (p=0.026, Cohen’s d: -0.719; 95% CI: -1.35 to -0.09). Elevation of high-density lipoprotein was observed in pooled estimate with p=0.016 and Cohen’d: 1.34 (95% CI: 0.25 to 2.44). Qualitatively, significant reduction of HbA1C, total cholesterol, and C-reactive protein were reported in at least two trials. Significantly improved quantitative insulin sensitivity check index (QUICKI) and decreased malondialdehyde, fructosamine, and fasting blood glucose were reported in at least one trial each. There were conflicting results on the change of low-density lipoprotein level. This study highlights that vitamin D supplementation promotes wound healing process among DFU patients; however, it is too premature to draw solid conclusions as the efficacy could be affected by multiple factors. Therefore, clinical trials from various demographics and ethnicities by using a high- versus low-dose model are needed.

Keywords: Diabetes complication, diabetic foot, wound healing, vitamin D, calcitriol
**Introduction**

Diabetes remains a significant public health problem worldwide, with approximately 529 million people, or a prevalence of 6.1% of all ages [1]. Diabetes leads to a constellation of complications, and diabetic foot ulcer is one of the most typical findings as the consequences of diabetic peripheral neuropathy developed from microvascular damage, metabolic alterations, and persistent inflammatory status in the axon [2]. DFU prevalence shows wide variations geographically, ranging from 3.0% to 13.0% in Oceania and North America, respectively. In the population with diabetes, the annual incidence of DFU is between 1.9% to 4.0% but can be higher in patients with established neuropathy [3]. The complication substantially leads to poor outcome in patients with diabetes, where previous study revealed a 5-year mortality of 30.5% was contributed by this ailment [4]. Another study reported a nearly 50% mortality within 5 years with cardiovascular disease (CVD) and infection as the leading cause [5]. Risk factors for DFU development are copious, including advanced age, male, elevated body mass index (BMI), prolonged duration of disease, diabetes-associated comorbidity (nephropathy, neuropathy, retinopathy), and elevated systolic blood pressure [6].

Recent studies have investigated the possible involvement of vitamin D in the pathogenesis of diabetes and its related complication [7, 8]. This fat-soluble vitamin's receptor has been discovered in pancreatic beta cells, implicating a possible function in insulin secretion [7, 8]. Furthermore, insulin sensitivity and function have been linked to serum vitamin D levels [7]. Vitamin D has also been shown to affect gene expression, modulate inflammation and oxidative stress, while also interact with insulin signaling pathways [7, 9]. This fact shows that Vitamin D may improve the condition of diabetes, and therefore reducing the incidence of its complication including DFU [10]. Regardless, there are several discouraging evidence regarding the efficacy of vitamin D in preventing insulin resistance and diabetes mellitus type 2 among prediabetic patients [11].

Multiple studies have reported direct association of Vitamin D with DFU [11-13]. Vitamin D particularly has emerging role as antimicrobial and anti-inflammatory agents via production of antimicrobial peptide, inhibiting bacterial biofilm, and suppress the activation of T-cells [14-16]. Despite this, the evidence gained in the preceding investigations is limited. In this systematic review and meta-analysis, we aimed to further establish and explore evidence to confirm the association between vitamin D supplementation and DFU. In addition, meta-regression was performed to portray the heterogeneity contributors which could provide better trajectories for future research.

**Methods**

**Study design and registry**
The study used Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline. The research question for the present systematic review and meta-analysis was, “What is the efficacy of vitamin D supplementation in treating diabetic foot ulcer?” The protocol registration had been carried out on PROSPERO (CRD42023415750).

**Search strategy**
The literature search was performed on 10 March 2023, using the search engine from the following databases: PubMed, Scopus, Embase, Scilit, Sci-Finder, LILACS, EuropePMC, medRxiv, bioRxiv, Google Scholar, Semantic Scholar, and Garuda. Clinical trial databases, namely ClinicalTrials.gov, International Clinical Trials Registry Platform (https://trialssearch.who.int/), CENTRAL (https://www.cochranelibrary.com/search). Keywords “diabetes”, “Foot ulcer”, and “Vitamin D” as well as their respective synonyms were used in combinations as presented in Table 1.

**PICOS framework**
The inclusion of the studies was in accordance with PICOS framework (participant, intervention, control, outcome, and study design) as follows: (P) Patients with diabetes following the criteria...
by American Diabetes Association’s (ADA) guideline [17]; (I) Vitamin D supplement and standard care; (C) Receiving no supplement, placebo, or lower dose of vitamin D supplement; (O) Wound area, length, width, and/or depth; (S) Observational (retrospective/prospective) and randomized controlled trials (RCTs). Studies that recruited pregnant and lactating women were not included. Articles published as literature reviews, commentaries, editorials, case reports, erratum, and conference abstracts, were not included. Articles reporting the results from in-vivo and in-vitro studies were also excluded. The included studies were limited to those reporting in English or Indonesian language.

**Screening and selection of the records**

The screening as well as the selection process were performed independently by M.I and T.F.D. Duplication on the retrieved records from the database was removed automatically by Mendeley Desktop v1.19.8 (https://www.mendeley.com/). Thereafter, the title and abstract of each record were screened. Records passing the initial screening would undergo a full-text review in which the criteria for inclusion or exclusion were applied. Different results from these screening steps would be overcome by revisiting the article and the consensus would be reached by discussion. Consultation with another reviewer would be required if a consensus could not be reached.

**Critical appraisal**

Cochrane ‘risk-of-bias 2.0’ tool and Newcastle Ottawa Scale were used to assess the quality of RCTs and observational studies, respectively. This assessment was carried out by two independent reviewers – S.A. and I.Q. For RCTs, the studies were considered to have a high-risk bias if one of the domains was marked with high-risk. For the observational studies, only those receiving ≥ 7 score in Newcastle Ottawa Scale would be included in the meta-analysis.

**Data extraction**

Firstly, the first author’s name, year of publication, the study location (country), study design, and sample size were collected. Patients’ characteristics including the type of diabetes, age, sex, diabetes onset, body mass index, and glycated hemoglobin (HbA1c) level were extracted from each study. Moreover, the data collected also included dosage and duration of the vitamin D supplement, vitamin D level, HbA1c, ulcer length, width, and depth, erythrocyte sedimentation rate (ESR), plasma malondialdehyde (MDA), total cholesterol (TC), total glyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL, and C-reactive protein (CRP). Continuous data would be presented as mean±standard deviation (SD), while ordinal would be presented as frequency (%). Data presented as median were converted to mean±SD.

**Data analysis**

Quantitative analysis was performed on Cochrane Collaborations – RevMan version 5.4.1. (22) and Comprehensive Meta-Analysis version 3 software. Random-effects model was applied to overcome measurement. The effect of vitamin D supplementation on the diabetic foot ulcer was assessed by Cohen’s d. Statistical significance was assumed if $p<0.05$. Meta-regression was carried out under random-effect model for age, male-to-female ratio, and BMI. Risks of publication bias were assessed based on Begg’s funnel plot if the number of pooled studies was ≥ ten.

**Results**

**Studies’ characteristics**

The screening and selection were performed in multiple stages following the PRISMA guideline, where the summary of this process is presented in **Figure 1**. In the initial identification stage, we found 4441 studies from the databases and 13 studies from clinical trial registry. As many as 1055 studies were removed from automatic duplicate screening, and 3318 studies were further excluded in the title abstract screening based on relevance. Through the screening, we also found that two clinical trials did not report their results or have their results published. Full-text screening resulted in the exclusion of a total of 74 studies. Thirty-one studies were considered irrelevant to the systematic review topic. Twenty-one studies were excluded because they did not
perform vitamin D supplementation, only investigating the serum level \((i.e. \ [18-22])\). A study matched the eligibility criteria of the participants and intervention, but they did not report outcome related to foot ulcer (wound area, depth, etc.) \([23]\). Studies using other supplement as control or those without control were excluded \([24-26]\). Finally, five studies were included in this systematic review \([27-31]\).

Characteristics of the five included studies are presented in Table 1. The studies were reported from India \([27]\), Iran \([28]\), Iraq \([29]\), Iran \([31]\), Denmark \([30]\). Two studies were double-blind \([30, 31]\), two studies were single-blind \([28, 29]\), and one study was open-label \([27]\). Total number of participants were 123 in experimental group and 122 in control group, with 30 versus 30 as the highest number of participants in individual studies \([28, 30]\). Most of the studies had relatively balance proportion of men and women \([28, 29, 31]\), yet two others were predominated by men \([27, 30]\). Three studies recruited patients with grade II or III foot ulcer \([27-29, 31]\). Control groups in the included studies are varied, placebo was used in \([29, 31]\), standard care without supplement in \([27]\), lower dose of vitamin D supplements in \([28, 30]\).

![Figure 1. PRISMA flow-chart for the study selection based on the eligibility criteria](image)

**Quality of the included studies**

Three studies did not properly report the dropped-out participants, and how to handle the missing data \([27-29]\). Most of the study sufficiently reported the randomization procedure \([27, 28, 30, 31]\), except in one study \([29]\). Four studies were found to have no deviation from the intended intervention \([28-31]\), while there were some concerns of this issue in a single study \([27]\). In terms of measuring the wound area, we concern on the use of non-standardized method based on manual calculation \([27, 29]\). Three studies were considered low risk in reporting the results because they used standardized computer software to calculate the wound area or depth \([28, 30, 31]\). Overall, two studies were marked with high risk, one study – some concerns, and two studies – low risk (Figure 2).
<table>
<thead>
<tr>
<th>Author, Year [Ref]</th>
<th>Country</th>
<th>Type of study</th>
<th>Characteristics of the subjects</th>
<th>Intervention</th>
<th>Ulceration*</th>
<th>Secondary outcomes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kamble et al., 2020 [27]</td>
<td>India</td>
<td>Open-label RCT</td>
<td>n</td>
<td>Age, years old Male/Female DFU grade BMI, kg/m²</td>
<td>Oral vit D (60,000 IU) for 12 weeks</td>
<td>Area (↓); width (↓); SVD (↑), HbA1c (↓), HDL (↑), TG, TC (↓)</td>
</tr>
<tr>
<td>Mozaffari-Khosraviet et al., 2016 [28]</td>
<td>Iran</td>
<td>Single-blind RCT</td>
<td>n</td>
<td>Age, years old Male/Female DFU grade BMI, kg/m²</td>
<td>Oral vit D (300,000 IU) Oral vit D (150,000 IU)</td>
<td>Area (↓) SVD (↑), FBG (↓), CRP (↓), ESR (↓), WBC (↓)</td>
</tr>
<tr>
<td>Rahman et al., 2013 [29]</td>
<td>Iraq</td>
<td>Single-blind RCT</td>
<td>n</td>
<td>Age, years old Male/Female Onset, years DFU grade BMI, kg/m²</td>
<td>Vit D 1000 IU twice daily for 4 weeks</td>
<td>Placebo Area (↓) Fructosamine (↓), LDL, HDL</td>
</tr>
<tr>
<td>Razzhagi et al., 2017 [31]</td>
<td>Iran</td>
<td>Double-blind RCT</td>
<td>n</td>
<td>Age, years old Male/Female DFU grade BMI, kg/m²</td>
<td>Oral VD (50,000 IU) every 2 weeks for 12 weeks</td>
<td>Placebo Length (↓), width (↓), depth (↓) SVD (↑), insulin (↓), QUICKI (↑), HOMA-IR, HbA1c (↓), LDL (↓), HDL, TG, TC (↓), MDA, (↓), CRP (↓), TAC, GSH</td>
</tr>
<tr>
<td>Halschou-Jensen et al., 2021 [30]</td>
<td>Denmark</td>
<td>Double-blind RCT</td>
<td>n</td>
<td>Age, years old Male/Female DFU grade BMI, kg/m²</td>
<td>High dose VD (170 μg/day) for 48 weeks or until ulcer resolution</td>
<td>Ulcer area (↓)</td>
</tr>
</tbody>
</table>

BMI, body mass index; CRP, C-reactive protein; DFU, diabetic foot ulcer (DFU grade was based on Wagner’s classification); ESR, Erythrocyte sedimentation rate; FBG, fasting blood glucose; GSH, total glutathione; HDL, high-density lipoprotein; HOMA-IR, homeostasis model of assessment—estimated insulin resistance; LDL, low-density lipoprotein; MDA, malondialdehyde; QUICKI, quantitative insulin sensitivity check index; RCT, randomized controlled clinical trial; TAC, total antioxidant capacity; VD, vitamin D; WBC, white blood cells.

* (↓) and (↑) indicate significant lower- and higher-changes, respectively, in experimental group as compared to placebo; otherwise, no significant difference was observed.
Figure 2. Traffic light plot for the results of Cochrane risk of bias 2.0 assessment.

**Main outcomes**

Pooled estimate of the ulcer improvements (n=123 versus 122 for experimental and control, respectively) are presented in Figure 3. In this pooled analysis, wound area data were extracted from four studies [27-30], while in one study, the wound depth was collected [31]. Due to this heterogeneity in outcome, pooled estimate based on Cohen’s d was employed. It was found that the vitamin D supplementation has a significant benefit to attenuate the ulceration ($p<0.001$, Cohen’s d: 2.72). The 95% CI ranged from 1.02 to 4.42, suggesting that the positive outcome in all studies. The highest Cohen’s d was contributed by Rahman et al. (2013) with a value of 8.53, followed by Razzhagi et al. (2017) – 4.94. Meanwhile, Kamble et al. (2020), Mozzafari-Khosravi et al. (2020), Halschou-Jensen et al. (2017) had Cohen’d values less than 1 (0.85, 0.52, and 0.42, respectively).

Figure 3. Forest plot for the efficacy of vitamin D supplementation in improving diabetic foot ulcer.

**Sensitivity of the main outcome pooled estimates**

Sensitivity test based on leave-one-out method was performed on the main outcome to evaluate the robustness of the data. Forest plot generated from this analysis is presented in Figure 4. The overall Cohen’s d value remained positive when each of the five studies was removed, suggesting the robustness.

**Secondary outcomes**

**Serum vitamin D**

Pooled estimate for the effect of vitamin D supplementation on its serum level is presented in Figure 5. Increased in serum level of vitamin D was found to be statistically significant following the intervention ($p=0.026$, Cohen’s d: -0.719 [95% CI: -1.35 to -0.09]).
Figure 4. Forest plot for the leave-one-out analysis on the efficacy of vitamin D supplementation in improving diabetic foot ulcer. Otherwise stated the control group receive placebo or no vitamin D supplementation. *High versus low dose.

Figure 5. Forest plot for the effect of vitamin D supplementation on serum vitamin D level among diabetic foot ulcer patients. Otherwise stated the control group receive placebo or no vitamin D supplementation. *High versus low dose.

**Insulin and glycemic control parameters**

Pooled estimate was not performed on these parameters because of the insufficient data (same parameter only reported by no more than two studies). Therefore, it is best to analyze the data in a qualitative manner. Improved glycemic control as indicated by significant reduction of HbA1C after the vitamin D supplementation was reported by Kamble et al. (2020) [27] and Razzhagi et al. 2017 [31]. Additionally, indication of improved glycemic control based on serum fructosamine following the supplementation was reported by Rahman et al. 2013 [29]. Higher score of quantitative insulin sensitivity check index (QUICKI) was found in experimental group, but the score of homeostasis model of assessment-estimated insulin resistance (HOMA-IR) was not significantly changed [31]. Fasting blood glucose (FBS) was significantly reduced in experimental group [28].

**Cholesterol**

Qualitatively, there is a dispute about the effect of vitamin D supplementation on low-density lipoprotein (LDL). Razzhagi et al. (2017) reported the LDL was reduced significantly in intervention group [31], but no statistical change of LDL was observed by Rahman et al. (2013) [29]. Two studies reported similar results that no significant change observed in TG, while significantly decreased total cholesterol was observed in the experimental group [27, 31].

Only high-density lipoprotein (HDL) level was reported by at least three studies. We then performed pooled estimate for the effect of vitamin D supplementation on high density lipoprotein (HDL) level, where the forest plot is presented in **Figure 6**. The analysis revealed that the intervention could significantly reduce HDL levels with \( p=0.016 \) and Cohen’s d: 1.34 (95% CI: 0.25 to 2.44).
Figure 6. Forest plot for the effect of vitamin D supplementation on HDL level among diabetic foot ulcer patients

**Inflammatory parameters**
Two studies reported the significant reduction of C-reactive protein (CRP) [28, 31]. Other inflammatory-related parameters such as erythrocyte sedimentation rate (ESR) and white blood cells (WBC) were not significantly different between the experimental and control groups [28]. A study found that malondialdehyde (MDA) was significantly reduced due to the intervention, but no change was observed in total antioxidant capacity (TAC) and total glutathione (GSH) [31].

**Meta-regression**
The meta-regression was performed to identify the factors correlated with the efficacy of vitamin D supplementation in diabetic foot ulcer, and the bubble plots for this analysis are presented in Figure 7. Age and BMI were not correlated with the vitamin D efficacy (p > 0.05). Male-to-female ratio, however, was found to be significantly correlated with the supplementation efficacy (p < 0.001).

Figure 7. Bubble plot for the correlations of age (a), body mass index (b), and male-to-female ratio (c) with the efficacy of vitamin D supplementation in diabetic foot ulcer management, respectively.
Discussion

Meta-analysis has been established for the association of diabetic foot ulcer with serum vitamin D [32]. It is therefore compelling if administering vitamin D as a part of DFU management could yield any benefits. In the present study, we included a total of five studies reporting such benefits [27–31]. The pooled analysis confirmed that vitamin D supplementation could increase the wound healing of diabetic foot. The supplementation also elevated its serum level, suggesting that DFU is indeed correlated with hypovitaminosis D. Herein, pooled analysis from three studies also suggest that vitamin D could improve hyperglycemic and dyslipidemic conditions, as suggested by the decrease in HbA1c and increase in HDL levels, respectively. This is in line with previous studies revealing the reduction of HbA1c associated with vitamin D supplements [33-35]. In previous meta-analysis, vitamin D has been found to appear beneficial in improving various diseases among diabetic individuals, namely nonalcoholic fatty liver disease [36, 37], diabetic kidney diseases [38], and diabetic peripheral neuropathy [39].

Previous reports suggest that supplementation of this vitamin has been found efficacious to promote wound healing [40]. Vitamin D is found to regulate the life cycles of keratinocytes, from their proliferation, differentiation, up to their apoptosis [41]. Additionally, the receptor of this vitamin is expressed in macrophage suggesting and receptor—vitamin D complex could act as the transcription factor for the production of antimicrobial peptides [42]. Moreover, the transcription factor also intertwines with immune cells signaling, as evidenced by its correlation with the serum levels of anti- and pro-inflammatory interleukins [21, 43]. In a mouse model, vitamin D, together with calcium, have been associated to interact with activation, migration, and differentiation of epidermal stem cells which consequently affect the re-epithelialization [44]. As vitamin D modulates the cascade of inflammatory response, it may promote the wound healing process via the attenuation of inflammation-caused disruption of endothelial colony-forming cells barriers [45]. Though vitamin D is prominent in wound healing, more complex underlying factors are involved in DFU.

DFU is a pathologic condition that is believed to be progressed from diabetic consequences such as peripheral arterial diseases (PAD) and systemic inflammation. In hyperglycemic conditions, production of reactive oxygen species (ROS) in mitochondria is increased [46, 47]. Overwhelming oxidative stress induces the activation of pro-inflammatory signaling pathways including nuclear factor-kappa B (NF-κB), mitogen-activated protein kinases (MAPKs), and inflammasome complexes [48]. In fact, in patients with DFU, the inflammatory factors are found to be elevated [22, 49-51]. This condition could further lead to endothelial damage and atherosclerotic plaque formation underlying the PAD [52]. Supplementation of vitamin D could restore these conditions by acting as inhibitors against the expression of pro-inflammatory cytokines and activation of nuclear factor-kappa B (NF-κB) [53, 54]. Further, vitamin D could scavenge the excessive ROS which eventually restores the oxidative stress balance, at least as suggested by an in-vivo study [55]. In a meta-analysis, vitamin D supplementations have been concluded to improve the oxidative stress parameters, though it remains unknown whether the effect is clinically significant [56]. More importantly, vitamin D is suggested to improve the insulin resistance and glycemic control which could stop the excessive blood glucose [7, 57, 58]. Taken altogether, vitamin D does not only promote wound healing in diabetic foot ulcer but also improves its underlying conditions.

Performing the pooled analysis for data presented in different parameters is challenging, but we overcome it by calculating the Cohen’s d instead. The results are, therefore, more difficult for comparison as opposed to mean difference or standardized means difference. However, using Cohen’s d resolves the variety in the data which could generate better robustness. Unfortunately, interpretation of the results from the present study should consider the poor quality of the data, as two studies were rated as high risk. Despite these findings, sensitivity test confirmed that vitamin D supplementation is efficacious in resolving DFU. As the limitation in performing the systematic review, we were unable to identify experts who performed the investigation on vitamin D supplementation for DFU management as this topic has not been widely investigated.
Conclusions

Vitamin D supplementation is efficacious in healing the wound of diabetic foot. The findings confirm the vital role of vitamin D in the development of DFU, and the supplementation could reverse the pathologic condition. Wound healing properties of vitamin D is associated with its activity in regulating immune response and prevent infections in the wound surface. Moreover, vitamin D has direct and indirect actions that contribute to the proliferation, differentiation, and apoptosis of keratinocytes. There are strong indications that vitamin D improves glycemic control and dyslipidemia which are the underlying causes of DFU.

It is obvious that further clinical trials to prove the efficacy of vitamin D supplementation in DFU management should be carried out. The future studies should be well-designed and employ robust image processing software to record the outcome. Studies from different countries and populations are encouraged since the efficacy of vitamin D is affected by ethnicity, sun exposure, skin color, dietary pattern, and genetic factors. By considering the efficacy and safety of vitamin D, and on the basis of ethical considerations, it is best to employ 'higher versus lower vitamin D supplement' design rather than using placebo as control.

Ethics approval

Not required.

Acknowledgement

The authors acknowledge the support from our research group members (Raisha Fathima and Arita Yuda Katiara Rizki) during the study and the writing of this work.

Funding

The authors received no external funding.

Competing interests

The authors declare that they have no known conflicts of interest related to the publication of this work.

Underlying data

All underlying data have been presented.

How to cite


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