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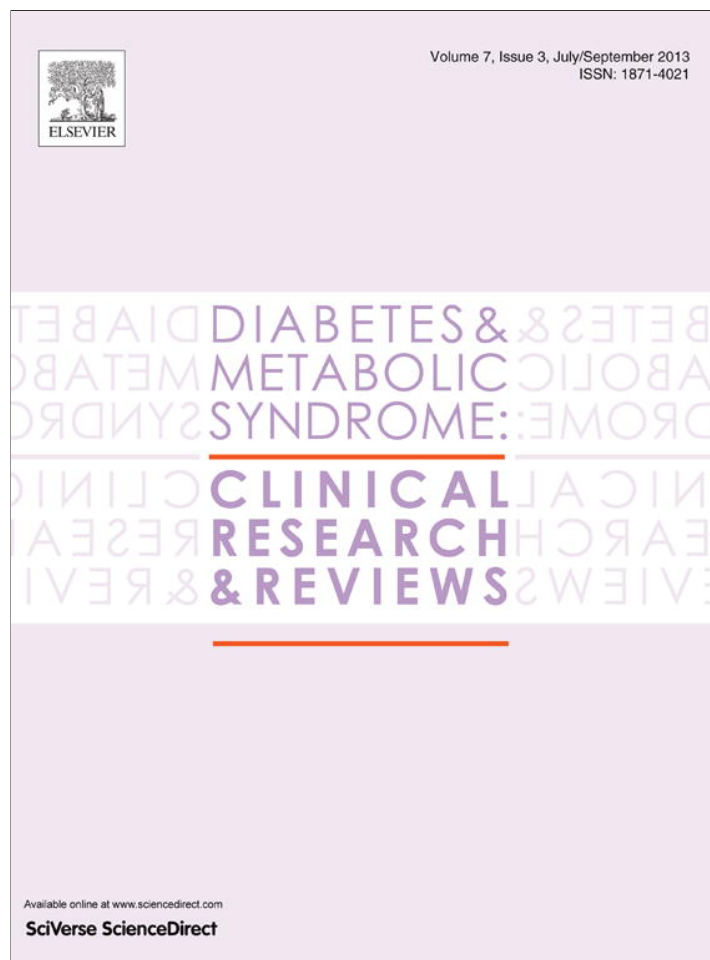
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25-Hydroxyvitamin D [25(OH)D] levels and diabetic foot ulcer: Is there any relationship?

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ABSTRACT

Aims: In recent years, there has been an effort to understand possible roles of 25(OH)D, including its role in the immune system particularly on T cell mediated immunity, pancreatic insulin secretion and insulin action. 25(OH)D stimulates the cell differentiation and reduces cell proliferation, which is essential for cell growth and wound healing. However, data on the association between low level of plasma 25(OH)D and diabetic foot syndrome are scarce.

Materials and Methods: Circulating plasma levels of 25(OH)D were measured in diabetic patients with ulcer ($n = 162$) and without ulcer ($n = 162$) in a prospective cohort hospital based study.

Results: Of these patients, 85.1% had type 2 diabetes. Subjects with diabetic foot ulcer showed lower median plasma level of 25(OH)D [6.3(4.2–11.1) vs 28.0(21.4–37.0)] ng/ml after adjusting the age and BMI. Regardless of the low levels of 25(OH)D in cases and controls, it was associated with neuropathy, sex (female), duration of ulcer healing, and smoking status and independent of confounding factors, including BMI (kg/m^2), A1c (%), hypertension, nephropathy, foot ulcer, retinopathy, CAD, PAD, HDL-C (mg/dl) and LDL-C (mg/dl). The factors which predict the risk of developing ulcer independent of 25(OH)D status were A1c ($>6.9\%$) [OR 4.37; RR 1.77], HDL-C (<40 mg/dl) [OR 1.16; RR 1.07], LDL-C (>100 mg/dl) [OR 1.07; RR 1.03], triglycerides (>200 mg/dl) [OR 1.40; RR 1.19], neuropathy [OR 6.88; RR 3.12], retinopathy [OR 3.34; RR 1.91], hypertension [OR 1.64; RR 1.28], nephropathy [OR 3.12; RR 1.87] and smoking [OR 4.53; RR 2.99] using odds and risk ratios.

Conclusion: It is not clear whether the suppression of delayed wound healing seen during 25(OH)D deficiency is due to the secondary effect or is a direct action of vitamin D on certain components of the immune system. Long-term randomized trials are needed to see the impact of vitamin D supplementation on the outcome of diabetic foot patients.

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1. Introduction

Infected diabetic foot ulcer (DFU) is a common cause of morbidity, ultimately leading to dreaded complications like gangrene and amputations. The life time risk of diabetic patients for development of a chronic foot wound has been estimated to reach 15–25% [1]; and despite considerable International efforts, DFU continues to be responsible for a high number of lower limb amputations that are associated with a substantial decrease in quality of life and increased risk of morbidity [2]. The major risk

factors for DFU are diabetic polyneuropathy and peripheral arterial disease (PAD) [3]. Infections are most often a consequence of foot ulceration, which typically follows trauma to a neuropathic foot [3]. These infections are usually polymicrobial and include aerobic gram-positive cocci (*Staphylococcus aureus*), gram-negative bacilli (*Escherichia coli*, *Klebsiella* sp., and *Proteus* sp.), and anaerobes (*Bacteroid* sp., and *Peptostreptococcus* sp. [2–6]).

The different phases of wound healing and their disturbances interfere with tissue homeostasis and wound healing are the crucial anti- and pro-inflammatory processes. Vitamin D has been shown to have numerous non-skeletal effects, including an important role in pancreatic insulin secretion and insulin action [7]. Although several studies have reported a protective relationship between vitamin D and the risk of developing diabetes, the data are not consistent. A meta-analysis found that three of six observational studies found an association between low vitamin D status and increased risk of incident type 2 diabetes or metabolic syndrome [8]. In contrast, eight clinical trials found vitamin D

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supplementation had no effect on glycemia or incident diabetes [7]. It may be that higher doses of vitamin D than those tested in clinical trials may be required to affect diabetes risk. Alternatively, the associations of calcium and vitamin D intake with improved glucose metabolism reported in observational studies may be the result of confounding by other components of foods containing these nutrients [9], outdoor exercise associated with solar radiation, or other factors [10,11]. To our knowledge, no study evaluated the role of 25(OH)D in patients with diabetic foot in comparison with diabetic subjects without foot complications from this part of world. Therefore, the aim of this study was to evaluate plasma levels of 25(OH)D in subjects with diabetic foot in comparison with subjects without foot complications.

2. Materials and methods

2.1. Study design

This was a prospective cohort hospital based-study. In total, 162 diabetic patients (group A) admitted in the Rajiv Gandhi Centre for Diabetes and Endocrinology, of Jawaharlal Nehru Medical College Hospital, Aligarh Muslim University, Aligarh, India who had ulcer or ulcers in their foot during the period December 2008–March 2011 were included. We also included 162 diabetic patients without foot ulceration (group B) admitted to our endocrine wards for other causes between 2009 and 2011. All patients gave informed consent to take part in this research. The study was carried out in accordance with principles of the Declaration of Helsinki as revised in 2001. Foot ulcer was defined as a full-thickness skin defect that required ≥ 14 days for healing [12]. Every subject with diabetic foot was matched for age (± 3 years), sex, BMI and blood pressure. Patients with inflammatory or infectious diseases, autoimmune and rheumatic diseases, cancer, haematological diseases, severe renal or liver failure, as well as those who were under treatment with anti-inflammatory drugs, were excluded. We also excluded patients with recent venous thromboembolism.

2.2. Clinical evaluation

A detailed history and physical examination were adopted from our previous studies [4].

2.3. Sample collection and determination of 25-hydroxyvitamin D levels

Serum samples were collected and kept frozen at -80°C until assay analysis. Serum levels of 25(OH)D by RIA method (DiSorin). Regarding the sensitivity of 25(OH)D, the analytical limit of detection was 4 ng/ml; intra- and inter assay coefficient of variation (CV%) were 5.2 and 7.4 respectively.

2.4. Statistical methodology

The results were analysed using the Sigma Plot Version 11.1 program. The Shapiro–Wilk test was used to evaluate normality of variables. The differences between the groups were calculated with Student *t* or the nonparametric *U*-Mann–Whitney tests. Results are expressed as median (lower quartile \leftrightarrow upper quartile) for continuous variables and percentages for categorical data, with $p < 0.05$ considered significant. A logistic forward regression analysis, multiple linear regression and chi square were used to assess the association between all clinical variables and inflammatory parameters that independently predicted foot ulcer development with a $p < 0.05$. Risk for ulcer development was also estimated by odds ratios (OR) and risk ratio (RR) with 95% confidence intervals (CIs) that independently predict the foot ulcer. Receiver operating

characteristics (ROC) curve was also analysed between patients with foot ulcer and without foot ulcer for 25(OH)D.

3. Results

3.1. Patients and foot characterizes

Baseline characteristic of subjects with diabetic foot in comparison with subjects without diabetic foot are given in Table 1. In group A, 68.7% of subjects were male, while 67.7% of subjects in group B were male. In group A, 86.6% of subjects had diabetes mellitus type 2, while in group B, type 2 diabetes was present in 85.5% of subjects. Regarding the duration of diabetes, 42.1% of subjects in group A vs 33.1% of subjects in group B could be diabetic by >10 years; the 57.8% vs 66.8% for <10 years in respective groups. The 29.3% of subjects in group A vs 44.7% of subjects in group B were treated with insulin, 41.7% vs 20.1% were in treatment with oral anti-diabetic, 28.9% vs 35.0% were in treatment with both insulin and oral anti-diabetic. 72.5% of subjects in group A vs 58.1% of subjects in group B were a smoker, 78.1% vs 37.9% had hypertension, and 56.8% vs 31.2% showed neuropathy. Retinopathy in group A was 67.7% vs 25.4% in group B, and 58.2% vs 22.5% had nephropathy respectively. Subjects in group A also presented, in comparison with those in group B,

Table 1
General and demographic variables in cases and controls.

Factors	Patients with ulcer 162	Patients without ulcer 162	<i>p</i> value
Age (years)	46.29 \pm 13.19	47.10 \pm 12.13	NS
T2DM/T1DM	134(82.7)/28(17.28)	146(90.1)/16(9.8)	NS
Male/female	103(63.5)/59(36.4)	102(62.9)/58(37.0)	NS
<i>Duration of diabetes</i>			
<10 years	111(68.5)	123(75.9)	NS
>10 years	51(31.4)	39(24.0)	0.048
Smoking (yes/no)	142(87.6)/52(32.0)	73(45.0)/43(54.9)	0.005
BMI (kg/m ²)	24.84 \pm 4.54	24.03 \pm 4.23	<0.001
Systolic BP (mmHG)	130.46 \pm 18.32	133.33 \pm 18.82	NS
Diastolic BP (mmHG)	85.24 \pm 16.8	86.34 \pm 11.36	NS
HbA1c (%)	9.6 \pm 2.03	7.9 \pm 0.86	<0.005
Plasma creatinine (mg/dl)	1.24 \pm 0.56	1.11 \pm 0.52	0.032
LDL-C (mg/dl)	75.89 \pm 18.34	104.38 \pm 30.1	<0.001
HDL-C (mg/dl)	34.60 \pm 3.34	44.30 \pm 7.7	<0.005
Total cholesterol (mg/dl)	136.93 \pm 13.7	181.9 \pm 32.3	<0.005
Triglycerides (mg/dl)	95.96 \pm 21.7	157.01 \pm 83.1	<0.005
Neuropathy	82(50.6)	47(29.0)	<0.0001
Retinopathy	82(50.6)	38(23.4)	<0.0001
Nephropathy	72(54.4)	31(19.13)	<0.0001
Hypertension	92(56.7)	67(41.3)	<0.0001
<i>Therapy</i>			
Insulin	62(38.2)	93(57.4)	<0.05
OHA	87(53.7)	42(25.9)	<0.005
Both	55(33.9)	73(45.0)	<0.005
<i>Grade of ulcer</i>			
Grade 1	48(29.6)	–	–
Grade 2	94(58.0)	–	–
Grade 3	20(12.3)	–	–
<i>Ulcer size</i>			
<4 cm ²	38(23.4)	–	–
>4 cm ²	124(76.5)	–	–
<i>Bacterial infection type</i>			
Superficial	48(29.6)	–	–
Subcutaneous	94(58.0)	–	–
Osteomyelitis	20(12.3)	–	–
Amputation	46(28.3)	–	–

Data are mean \pm sd or *n* (%) unless otherwise indicated. LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol.

Table 2

Systemic immune mediated concentrations in patients with and without foot ulcer in diabetic patients.

Immune mediators	DFU patients	DC patients	p
Serum 25(OH)D (ng/ml)	8.4(7.1–9.2)	29.8 (15.6–44.2)	<0.005

increased mean ± sd levels of HbA1c % [9.6 ± 2.14% vs 7.4 ± 0.86], BMI (kg/m²) [26.71 ± 1.26 vs 26.89 ± 1.12], serum creatinine (mg/dl) [1.67 ± 0.56 vs 1.11 ± 0.522], LDL-C (mg/dl) [77.87 ± 19.34 vs 104.97 ± 32.1], HDL-C (mg/dl) [34.87 ± 2.31 vs 44.37 ± 7.2], total cholesterol (mg/dl) [139.76 ± 12.7 vs 181.9 ± 32.3], and triglycerides (mg/dl) [97.93 ± 21.7 vs 142.12 ± 72.1] (Table 1). Regarding the prevalence of previous vascular morbidity, 14.6% of subjects in group A vs 11.5% of subjects in group B had PAD, 17.5% vs 10.0% had ischaemic heart disease. Finally, subjects of group A showed lower median serum level of 25(OH)D [6.3(4.2–11.1) vs 28.0(21.4–37.0)] ng/ml (Table 2).

3.2. Univariate analysis

On univariate analysis, the factors which showed a positive association in predicting the foot ulcer were HbA1c (>6.9%) [OR 4.37, RR 1.77], neuropathy [OR 6.88, RR 3.12], retinopathy [OR 3.34, RR 1.91], hypertension [OR 1.64, RR 1.28], nephropathy [OR 3.12, RR 1.87], smoking [OR 4.53, RR 2.99], HDL-C (<40 mg/dl) [OR 1.16, RR 1.07], LDL-C (>100 mg/dl) [OR 1.07, RR 1.03], triglycerides (>200 mg/dl) [OR 1.40, RR 1.19]. In a chi square test, the predictive factors were HbA1c (>6.9%) [p < 0.0001], total cholesterol (>150 mg/dl) [p < 0.0001], neuropathy [p < 0.0001], retinopathy [p < 0.0001], hypertension [p = 0.03], nephropathy [p < 0.0001] and smoking [p < 0.0001].

3.3. Multivariate analysis

The factors which showed a positive association in predicting the foot ulcer by multiple linear regression, forward stepwise regression analysis and one way ANOVA were total cholesterol (>150 mg/dl) [p < 0.001, p < 0.001, NS], triglycerides (>200 mg/dl) [p = 0.014, p = 0.038, NS], neuropathy [p = 0.002, p = 0.003, p < 0.005], retinopathy [p = 0.013, p = 0.007, p < 0.05], hypertension [p = NS, p < 0.005, p < 0.05], nephropathy [p < 0.007, p < 0.003, p < 0.05] (Table 3). Low plasma 25(OH)D (ng/ml) [p < 0.001, p < 0.001, p < 0.05] showed a correlation in predicting the foot ulcer by multiple linear regression and forward stepwise

Table 3

Chi square test, odds ratio, risk ratio, multiple linear regression analysis, forward stepwise regression analysis and one way ANOVA analysis to study the independent variable predicting foot ulcer in diabetic patients.

Independent variable	Odds ratio	Risk ratio	Chi square test		Multiple linear regression	Forward stepwise regression analysis	One way ANOVA
	OR(95%CI)	RR(95%CI)	YATES	p			
HbA1c (>6.9%)	4.37(2.33–8.19)	1.77(1.46–2.15)	22.25	<0.0001	NS	NS	<0.05
Total cholesterol (>150 mg/dl)	0.18(0.06–0.17)	0.28(0.20–0.40)	80.94	<0.0001	<0.001	<0.001	NS
Triglycerides (>200 mg/dl)	1.40(0.86–2.28)	1.19(0.91–1.54)	1.54	0.216	0.014	0.038	NS
HDL-C (<40 mg/dl)	1.16(0.71–1.87)	1.07(0.84–1.38)	0.24	0.624	NS	NS	<0.05
LDL-C (>100 mg/dl)	1.07(0.69–1.67)	1.03(0.83–1.29)	0.05	0.823	NS	NS	DNT
Neuropathy	6.88(3.96–11.9)	3.12(2.10–4.67)	51.24	<0.0001	0.002	0.003	<0.05
Retinopathy	3.34(2.07–5.38)	1.91(1.44–2.55)	24.47	<0.0001	0.013	0.008	<0.05
Hypertension	1.64(1.05–2.54)	1.28(1.02–1.59)	4.46	0.03	NS	<0.001	<0.05
Nephropathy	3.12(1.91–5.11)	1.87(1.38–2.53)	20.35	<0.0001	<0.007	0.003	<0.05
Smoking	4.53(2.77–7.41)	2.99(1.68–3.31)	37.18	<0.0001	NS	NS	DNT

The following independent variable were considered for the model: Serum 25(OH)D (ng/ml), BMI (>25 kg/m²), HbA1c (>6.9%), total cholesterol (>150 mg/dl), triglycerides (>200 mg/dl), HDL-C (<40 mg/dl), LDL-C (>100 mg/dl), neuropathy, retinopathy, hypertension, smoking. Only the variable that had a p value <0.05 were considered in the final fitted model. NS: non-significant, DNT: Do not test.

* Shapiro–Wilk test.
 ** Shapiro–Wilk test.
 *** Kolmogorov–Smirnov test.

Table 4

Multiple linear regression analysis, forward stepwise regression analysis and one way ANOVA analysis to study the independent variable predicting foot ulcer in diabetic patients.

Independent variable	Multiple linear regression		Forward stepwise regression analysis		One way ANOVA
	R ²		R ²		
	Coefficient	p-value*	p-value**	p-value***	
Serum 25(OH)D (ng/ml)	-0.0046	<0.001	<0.001		<0.05

The serum 25(OH)D (ng/ml) was considered as an independent variable for the model. Only the variable that had a p value <0.05 were considered in the final fitted model.

* Shapiro–Wilk test.
 ** Shapiro–Wilk test.
 *** Kolmogorov–Smirnov test.

Table 5

Correlation analysis between 25(OH)D levels and laboratory and clinical variables in patients with diabetic foot.

Independent variable	Serum 25(OH)D (ng/ml)	
	r	p
Ulcer Grade	0.012	0.002
Total cholesterol (>150 mg/dl)	0.032	0.684
Triglycerides (>200 mg/dl)	0.010	0.893
HDL-C (<40 mg/dl)	0.017	0.829
LDL-C (>100 mg/dl)	0.106	0.177
Neuropathy	0.085	0.007
Retinopathy	-0.019	0.812
Hypertension	-0.036	0.004
Nephropathy	0.007	0.925
Smoking	0.107	0.03
Infection type (superficial, subcutaneous, osteomyelitis)	0.106	0.007
Amputation	0.074	0.04
ESBL infection	0.158	0.006
MRSA infection	0.084	0.02

The following independent variable were considered for the model: Serum 25(OH)D (ng/ml), BMI (>25 kg/m²), Serum hsCRP (mg/ml), total cholesterol (>150 mg/dl), triglycerides (>200 mg/dl), HDL-C (<40 mg/dl), LDL-C (>100 mg/dl), neuropathy, retinopathy, hypertension, smoking, ESBL, extended spectrum beta lactamases, MRSA, methicillin resistant *S aureus*. Only the variable that had a p value <0.05 were considered in the final fitted model.

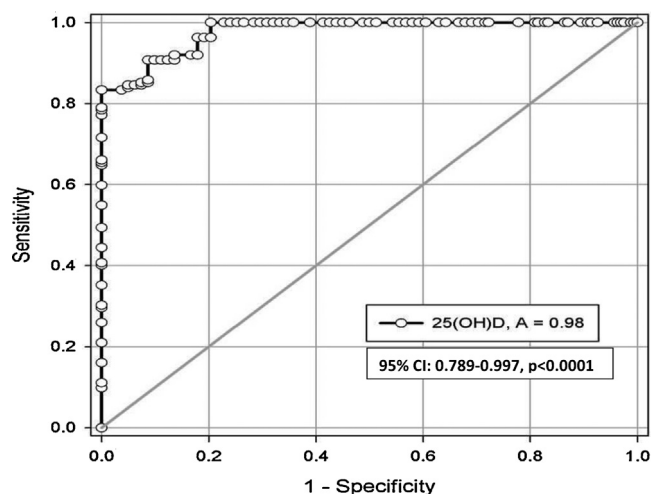


Fig. 1. Receiver operation characteristic curve of plasma 25(OH)D in diabetic patients with/without foot ulcer. 95% CI: 0.789–0.997, $p < 0.0001$.

regression (Table 4). According to ROC analysis, the curve area (95% CI and p value was significant when group A patients compared with group B patients for low serum 25(OH)D ng/ml) [0.986(0.789–0.997), $p < 0.0001$] (Table 5 and Fig. 1).

3.4. Correlation analysis

A significant correlation corrected for age and BMI, between 25(OH)D and ulcer grades [$r = 0.012$, $p < 0.002$], A1c ($>6.9\%$) [$r = 0.342$, $p < 0.032$], neuropathy [$r = 0.085$, $p = 0.007$], hypertension [$r = -0.036$, $p < 0.004$], smoking [$r = -0.003$, $p = 0.004$] infection type [$r = 0.106$, $p = 0.007$], amputation [$r = 0.074$, $p = 0.04$], ESBL infection [$r = 0.158$, $p = 0.006$] and MRSA infection [$r = 0.084$, $p = 0.02$] was also seen.

4. Discussion

In this study, we have demonstrated that diabetic patients with foot ulcer in comparison with diabetes without diabetic foot have an low circulating levels of serum 25(OH)D. Prevalence of severe 25(OH)D deficiency was remarkably higher in cases than in controls similar to recent findings of Tiwari et al., [13]. Foot infection in patients with diabetes reflects their poor immune status compared to patients with diabetes. These associations were present when age (± 3 years), sex and BMI (± 2 kg/m²) were matched and co-morbidities were taken into account in a univariate, multiple regression and ROC analysis. The severity of DFU based on University of Texas was associated with circulating levels of serum 25(OH)D.

Researchers have linked vitamin D with several other immunological alterations that are associated with increased susceptibility towards infection. It has also been shown that active vitamin D3 stimulates the phagocytosis and killing of bacteria by macrophages [14] and is a potent suppressor of interferon-g-mediated macrophage activation [15]. It suppresses T cell proliferation and decreases the production of the T helper type 1 cytokines while promoting the production of T helper type 2 cytokines [16]. T helper type 2 cells primarily play a role in response to extracellular pathogens (most bacteria and parasites). In addition to hyperglycaemia, deficiency of vitamin D might also increase the risk of infection in diabetic foot patients by further depleting the immune cells' response against infection.

Our study showed that vitamin D deficiency may be related to the development of foot ulcer in diabetes. The effects of vitamin D deficiency on the cardiovascular system may be very important.

Vitamin D receptors are found in many cells of the cardiovascular system. Several plausible mechanisms explain how vitamin D may modify the risk for cardio-metabolic outcomes. In many studies, Vitamin D regulates the renin–angiotensin system, suppresses proliferation of vascular smooth muscle, improves insulin resistance and endothelial cell-dependent vasodilation, inhibits anti-coagulant activity and myocardial cell hypertrophy, and may modulate macrophage activity and cytokine generation [17,18]. Clinical studies have associated low levels of vitamin D with hypertension, coronary artery calcification, and cardiovascular diseases such as myocardial infarction, acute stroke, and congestive heart failure [19–21]. In addition, vitamin D deficiency has also been associated with the increased incidence of cardiovascular disease in the Framingham offspring study [22]. Meta-analysis of vitamin D and cardiometabolic outcomes reported lower vitamin D status in healthy adults was associated with increased risk for hypertension and possibly cardiovascular disease. However, data on associations between vitamin D status and cardiometabolic outcomes in type 2 diabetes were unclear. Hypovitaminosis D appears to increase the risk of developing T2DM [23]. Such relationship between vitamin D and T2DM is predictable given that vitamin D deficiency has been associated with insulin resistance and impaired β cell function [23–25]. Recent evidence from the Japanese population suggests an association between 1,25-dihydroxy vitamin D deficiency and the presence of microvascular complications in T2DM [26,27]. In addition, there is an inverse association between vitamin D concentration and CVD events in patients with T2DM and renal impairment [28]. There is also a strong inverse association between serum 25(OH)D concentrations and carotid artery IMT, a marker of a preclinical atherosclerosis among type 2 diabetic patients. Our study showed higher prevalence of vitamin D inadequacy (97.1%) in DFU patients compared with diabetic patients.

In many previous studies, lower serum 25(OH)D levels were positively associated with prediabetes in U.S. adults. This association was independent of confounding factors, including age, sex, race/ethnicity, smoking, alcohol intake, BMI, physical activity, hypertension, systolic blood pressure, serum total cholesterol, CRP levels, and eGFR. Our results shows the effect of low serum 25(OH)D on diabetes by demonstrating for the first time that 25(OH)D levels are independently associated with foot ulcer in diabetes. Previous reports have shown that lower serum 25(OH)D levels are related to CVD and CVD mortality [29,30]. Several lines of recent evidence also suggest that 25(OH)D insufficiency may be involved in the risk of developing diabetes [24,31], a risk factor for CVD. In animal models, low levels of 25(OH)D have been shown to impair insulin synthesis and secretion, and treatment with 25(OH)D has, in turn, been shown to delay the onset of diabetes [32]. However, the results from human studies have not been similarly consistent [8]. First, although some epidemiologic studies have reported a positive association [23,33] between low serum 25(OH)D levels and diabetes, others did not find an association after multivariable adjustment [25,34] or in an analysis among women [35]. Pittas et al. [8], in a recent meta-analysis, concluded that evidence was insufficient to support an inverse association between 25(OH)D levels and diabetes. Also, to our knowledge, the association between serum 25(OH)D levels and DFU has not been previously examined. A low body mass index, low serum albumin, and weight loss are associated with an increased risk of pressure ulcers [36]. The relationship between malnutrition and ulceration, the vitamin D deficiency may play a role in ulcer development. There are very few studies that have looked into the role of specific nutritional deficiencies. Unfortunately, there is no evidence in the literature looking specifically at vitamin D and pressure ulcers. To our knowledge, this was the first study to demonstrate the possible

role of down-regulated vitamin D in plasma and diabetic ulceration. The possible reason for low circulating levels of 25(OH)D might actually be one of the causes for development of DFU in diabetic patients. DFU basically results in patients with poor glycemic control or those with unidentified diabetes. DFU development involves long term uncontrolled diabetic pathogenesis and is thus generally found in patients with long diabetic history. It has already been found that patients with diabetes become vitamin D deficient over a period of time and that supplementation of vitamin D is generally given as an add-on therapy in order to avoid any complications [37]. From a pathophysiological standpoint vitamin D serves more than one purpose, it enhances both insulin secretion from pancreatic islets and insulin sensitivity in the peripheral tissues. Studies have reported the presence of vitamin D receptor (VDR) on pancreatic cells along with its expression on skeletal muscles and adipocytes, thus hinting towards the probable role of vitamin D in maintaining glucose homeostasis. When 1,25(OH)₂D interacts with the Vitamin D receptor (VDR) it induces heterodimerization of VDR with the retinoid X receptor (RXR). This heterodimer then binds to the Vitamin D response elements in DNA and recruits various coactivators leading to the enhanced transcription of genes whose protein products control calcium homeostasis [38] but the presence of rapid, transcription-independent events have also been observed in response to physiologic levels of vitamin D and the existence of 1,25(OH)₂D-inducible signal transduction pathways have been found within various tissues including adipose tissues [27] 1,25(OH)₂D rapidly (within seconds and minutes) stimulates events, normally associated with the activation of membrane receptors for growth factors and peptide hormones [39]. These include: (1) Phospholipase C (PLC) and phospholipase D activation. (2) Phosphoinositide turnover leading to the generation of the second messengers inositol 1,3,4-triphosphate (IP3) and 1, 2-diacylglycerol (DAG). (3) Increase in intracellular calcium by increasing calcium uptake and the release of intracellular calcium stores 4. Adenylate cyclase activation to increase cAMP levels and stimulation of protein kinase A (PKA) activity 5. Calcium-dependent protein kinase C (PKC) activation and cellular redistribution [40,41]. Vitamin D, thus in all probability may help in treating diabetes by influencing insulin sensitivity and secretion via its effects on intracellular calcium [24]. Vitamin D deficiency leads to a rise in PTH (parathyroid hormone) levels [42], which increases the intracellular calcium concentration [43]. Sustained elevations of intracellular calcium may inhibit both insulin secretion and insulin-target cells from sensing the brisk intracellular calcium fluxes necessary for insulin action, resulting in inhibition of GLUT1 and GLUT-2 in pancreatic cells and attenuating post receptor binding action of insulin and deactivation of GLUT-4 [44], the major glucose transporter in muscle and adipose tissues. On the other hand, pancreatic beta cells also depend on the intracellular calcium concentration for insulin secretion and therefore vitamin D supplementations to diabetic patients improves the insulin secretion and also increase the amount of glucose entering the tissues (including skeletal muscles). Therefore, vitamin D acts by removing high amount of glucose from circulation and also maintains its steady supply to tissues, hence fighting complications like DFU that basically arises due to prolonged exposure to higher than normal glucose levels which damages the nerves, causing peripheral neuropathy and ultimately resulting in DFU.

The strength of our study includes a relatively large study populations and the availability of additional data on the potential confounders so that multiple regression analysis were possible to analyse the impact of metabolic factor or co-morbidities on immune system. The only limitation was that, this study includes diabetic subjects with and without diabetic foot.

Summary, in this study, we strongly supports the hypothesis that lower serum 25(OH)D play an important role in the pathogenesis of foot ulceration, independent of BMI, sex, and age, however, further investigation of the underlying mechanisms is needed to elucidate this associations with comorbid conditions in DFU patients. The patients with foot ulcers exhibit a specific and non-random lower levels of serum 25(OH)D in the circulation, although these associations were markedly attenuated after multivariate analysis, however, these associations were independent of multiple potential confounders and were mainly associated with severity of ulceration (different grades of ulcer using University of Texas system) and microbial infection in foot. Further studies are needed to test whether this lower levels of serum 25(OH)D precedes the development of foot ulcer. Moreover, the characterization of beneficial and deleterious immune mediators in the process of wound healing in patients with ulcerations would be important to identify potential therapeutic targets and immunomodulating treatment options. Our data also raise the possibility that 25(OH)D might provide an adjunctive method for early detection of risk for foot complications in diabetes. It is possible to hypothesize on the participation of locally released markers in the development of DFU.

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