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Association of vitamin D status with all-cause mortality and outcomes among Chinese individuals with diabetic foot ulcers

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ABSTRACT

Aims/Introduction: The aim of this study was to examine the correlation between serum vitamin D concentrations and prognosis among Chinese individuals with diabetic foot ulcers (DFUs).

Materials and Methods: We retrospectively recruited 488 adults with DFUs in West China Hospital from 1 January 2012 to 31 December 2019. After telephone follow up, 275 patients were finally included. We compared serum vitamin D concentrations among DFUs patients with different prognostic status, and examined the association of vitamin D status with prognostic variables by Kaplan–Meier analysis. Cox proportional hazards models were used to estimate hazard ratios and 95% confidence intervals for all-cause mortality.

Results: The median concentration of serum vitamin D of patients with DFUs was 37.78 nmol/L (interquartile range 27.91–50.66 nmol/L), with 31.6% having vitamin D deficiency (<30 nmol/L) and 42.2% having insufficient vitamin D (<50 nmol/L). During a median follow-up period of 52 months, 65 patients died, with an all-cause mortality of 23.64%. Vitamin D deficiency was independently linked to increased all-cause mortality after multivariable adjustments (hazard ratio 0.565, 95% confidence interval 0.338–0.946, $P = 0.030$). There were no significant differences between vitamin D concentrations and other outcomes of DFUs. Patients who suffered amputations had a tendency of lower vitamin D concentrations (34.00 [interquartile range 26.90–41.81] vs 40.21 [interquartile range 29.60–53.96] nmol/L, $P = 0.053$).

Conclusions: Vitamin D deficiency was significantly associated with increased all-cause mortality in Chinese individuals with DFUs. Vitamin D supplementation might be a potential therapy for DFUs to prevent premature death and improve outcomes.

INTRODUCTION

In recent decades, the prevalence of diabetes has risen dramatically worldwide. As of 2019, approximately 463 million people were living with diabetes globally, which could increase to 592 million by 2035[1]. Diabetes-related complications impact not only life expectancy, but also quality of life, of which diabetic foot ulcer (DFU) is the most challenging complication[2]. Between 25 and 34% of diabetes patients might suffer from foot ulcerations during their lifetime[3]. The estimated recurrence rates of DFUs 1, 3 and 5 years after ulcer healing were 40, 60 and 65%, respectively[4]. Furthermore, once foot ulcers develop, the relative risk of all-cause death is approximately twice as high in people with diabetes as in individuals without foot ulcers[2]. Therefore, it is of great importance to identify and manage the potential risk factors for the prevention or postponement the onset of DFUs and its adverse outcomes.

Vitamin D, a pleiotropic steroid hormone that primarily regulates calcium and phosphate metabolism, as well as bone turnover, has been linked to glycemic control[5] and diabetes-related complications among diabetes patients[6]. In addition, vitamin D

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has been implicated in diabetic peripheral neuropathy[7,8] and peripheral arterial disease[9,10] which are frequently the most critical factors for the occurrence of DFUs[11]. Thus, the relationship between vitamin D and DFUs has become a topic of increasing concern. In fact, over recent years, accumulating evidence has shown the link between low vitamin D concentrations and the onset of DFUs[12,13]. However, to our knowledge, no studies focusing on the connection between vitamin D status and prognoses of DFUs have yet been reported, and there is still a very limited understanding about this respect.

To address these knowledge gaps, we carried out a retrospective study examining the relationship between serum vitamin D concentrations and the outcomes and all-cause mortality in a relatively large sample of Chinese adults with DFUs.

MATERIALS AND METHODS

Study population

In the present study, a total of 488 consecutive inpatients with DFUs were recruited between 1 January 2012 to 31 December 2019 at the Diabetic Foot Care Center, Department of Endocrinology and Metabolism, West China Hospital, Sichuan University (Chengdu, China). Participants were adults aged ≥18 years with type 2 diabetes mellitus and Wagner grade 1–5 foot ulcers. The exclusion criteria were as follows: (i) patients with non-diabetic ulcers, such as malignant ulcers, gouty ulcers and cryoglobulinemia-related ulcerations; (ii) patients with advanced liver cirrhosis or kidney disease, and refractory mental illness; and (iii) patients receiving glucocorticoids, immunosuppressive drugs or chemotherapy. This research protocol was approved by the Biomedical Research Ethics Committee of West China Hospital of Sichuan University (2018 [542]). Verbal informed consent was obtained from each participant.

Measurement of serum 25-OH-vitamin D

The serum total 25-OH-vitamin D concentrations were measured by the electrochemiluminescence immunoassay (Roche Cobas e601 analyzer; Basel, Switzerland) with a functional sensitivity of 10.03 nmol/L. According to the recommendations of the Institute of Medicine[14], the US Endocrine Society[15] and the latest evaluation results of vitamin D levels worldwide in 2020[16], in the present study, vitamin status was classified as sufficiency (25-OH-vitamin D ≥50 nmol/L), insufficiency (30 nmol/L ≤ 25-OH-vitamin D <50 nmol/L) and deficiency (25-OH-vitamin D <30 nmol/L).

Procedures

Demographics data, smoking status, glycated hemoglobin, duration of diabetes (years), wound duration (months) and Wagner grade were recorded. Telephone follow up was centralized for all recruited patients to assess their conditions after discharge, including wound healing, amputation, recurrence and death between January and February 2021. Healing is defined as complete epithelial cover in the absence of discharge[17]. Total healing rate and 12-week healing rate are expressed separately as the percentage of patients whose ulcers have healed by the end of follow up and within 12 weeks. Healing time is the time it takes for the wound to heal. Finally, 275 patients were successfully followed up, for a follow-up rate of 56.35%. The median time of follow up was 52 months (interquartile range [IQR] 26–72 months). Furthermore, only part of the data on wound healing, amputation, recurrence and death were available for some patients.

Statistical analysis

SPSS 18.0 software (SPSS, Chicago, IL, USA) was used for statistical analyses. Normally distributed continuous variables were represented by the mean and standard deviation. Non-normally distributed continuous variables were reported as the median and IQR (25–75%). Frequency counts were expressed as percentages (*n*/%). We carried out the statistical analysis with Student's *t*-test, Mann–Whitney *U*-test or χ^2-test, as appropriate.

Four outcome variables were selected for the analysis, including all-cause death, wound healing, recurrence and amputation. The serum vitamin D concentrations were categorized into different grades (deficiency, insufficiency or sufficiency) for Kaplan–Meier analysis to examine the association of vitamin D with prognostic variables. Differences in each outcome among groups were assessed using the log-rank test. In addition, unadjusted and adjusted Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence interval (CIs) for the association between vitamin D and all-cause mortality. A *P*-value <0.05 was considered statistically significant. Graphing was carried out using GraphPad Prism 7 software (San Diego, CA, USA).

RESULTS

Baseline characteristics

The baseline characteristics of the study population according to serum vitamin D status are shown in the Table 1. Among the 275 patients with DFUs, the mean age was 66.97 ± 9.96 years, and 66.9% of patients were men. The median concentration of serum vitamin D was 37.78 nmol/L (IQR 27.91–50.66 nmol/L); 31.6% had vitamin D deficiency (<30 nmol/L); 42.2% had insufficient vitamin D (<50 nmol/L); and 26.2% had sufficient vitamin D (≥50 nmol/L). There were no significant differences in body mass index, smoking status, glycemic control, the duration of diabetes and DFU, cardiovascular disease, education, residence and marital status among these groups. Participants who had lower vitamin D levels were more likely to suffer from Wagner grade ≥3 wounds ($P = 0.017$).

All-cause mortality

During the follow up of these 275 individuals, 65 deaths were documented and the all-cause mortality rate was 23.64%. Vitamin D concentrations were lower among patients who died than those who were alive (33.42 [IQR 23.32–46.88] vs 38.99 [IQR 29.00–52.48] nmol/L, $P = 0.006$). Three cumulative

Table 1 | Baseline characteristics of participants with diabetic foot ulcers

	Total (n = 275)	Vitamin D status			P
		Sufficiency (n = 72)	Insufficiency (n = 116)	Deficiency (n = 87)	
Sex					
Male	184 (66.9%)	52 (72.2%)	76 (65.5%)	56 (64.4%)	0.529
Female	91 (33.1%)	20 (27.8%)	40 (34.5%)	31 (35.6%)	
Age (years)	66.97 ± 9.96	66.08 ± 10.38	66.11 ± 9.43	68.86 ± 10.14	0.101
≤55	38 (13.8%)	11 (15.3%)	19 (16.4%)	8 (9.2%)	0.362
>55	237 (86.2%)	61 (84.7%)	97 (83.6%)	79 (90.8%)	
BMI (kg/m^2)	23.23 (21.53, 25.32)	23.36 (21.22, 25.82)	23.25 (21.79, 24.91)	23.21 (21.43, 25.78)	0.947
BMI <18.5	10 (3.6%)	3 (4.2%)	3 (2.6%)	4 (4.6%)	0.119
18.5 ≤ BMI < 24	151 (54.9%)	35 (48.6%)	63 (54.3%)	53 (60.9%)	
24 ≤ BMI < 28	85 (30.9%)	26 (36.1%)	42 (36.2%)	17 (19.5%)	
BMI ≥28	29 (10.5%)	8 (11.1%)	8 (6.9%)	13 (14.9%)	
Smoking status					
Smoking	135 (49.1%)	37 (51.4%)	57 (49.1%)	41 (47.1%)	0.867
Non-smoking	140 (50.9%)	35 (48.6%)	59 (50.9%)	46 (52.9%)	
Duration of diabetes (years)	12 (7, 19)	11 (6, 20)	12 (7, 19)	12 (7, 18)	0.976
25-(OH)-VD (nmol/L)		61.17 (53.14, 72.87)	38.69 (34.39, 42.83)	23.27 (19.32, 27.70)	
HbA1c (%)	7.80 (6.90, 9.40)	7.60 (6.93, 9.10)	7.90 (6.83, 9.48)	7.80 (6.70, 9.80)	0.760
eGFR (mL/min/1.73 m^2)	79.05 (56.11, 94.25)	83.06 (66.96, 96.48)	74.91 (55.87, 92.84)	75.84 (47.60, 92.40)	0.055
eGFR <30	16 (5.8%)	1 (1.4%)	5 (4.3%)	10 (11.5%)	**0.045**
30 ≤ eGFR < 60	57 (20.7%)	12 (16.7%)	26 (22.4%)	19 (21.8%)	
eGFR ≥60	202 (73.5%)	59 (81.9%)	85 (73.3%)	58 (66.7%)	
AST (IU/L)	20 (16, 29)	20 (15, 30)	22 (17, 29)	18 (16, 27)	0.400
ALT (IU/L)	19 (13, 29)	19 (11, 29)	21 (14, 29)	16 (12, 24)	0.130
Wound duration (months)	2.0 (1.0, 6.0)	1.0 (0.5, 5.0)	2.0 (1.0, 5.8)	2.0 (1.0, 6.0)	0.205
Wound classification					
Wagner grade 1	22 (8.0%)	11 (15.3%)	9 (7.8%)	2 (2.3%)	
Wagner grade 2	68 (24.7%)	25 (34.7%)	24 (20.7%)	19 (21.8%)	
Wagner grade 3	126 (45.8%)	25 (34.7%)	57 (49.1%)	44 (50.6%)	**0.017**
Wagner grade 4	53 (19.3%)	11 (15.3%)	22 (19.0%)	20 (23.0%)	
Wagner grade 5	6 (2.2%)	0 (0%)	4 (3.4%)	2 (2.3%)	
CVD	89 (32.4%)	20 (27.8%)	35 (30.2%)	34 (39.1%)	0.254
Education					
Less than high school	192 (69.8%)	53 (73.6%)	79 (68.1%)	60 (69.0%)	
High school or equal	49 (17.8%)	14 (19.4%)	20 (17.2%)	15 (17.2%)	0.61
College or above	34 (12.4%)	5 (6.9%)	17 (14.7%)	12 (13.8%)	
Residence					
Urban	116 (42.2%)	32 (44.4%)	49 (42.2%)	35 (40.2%)	
Villages and towns	75 (27.3%)	17 (23.6%)	31 (26.7%)	27 (31.0%)	0.889
Country	84 (30.5%)	23 (31.9%)	36 (31.0%)	25 (28.7%)	
Married	252 (91.6%)	65 (90.3%)	108 (93.1%)	79 (90.8%)	0.749
ABI					
>0.9	174 (63.3%)	52 (72.2%)	73 (62.9%)	49 (56.3%)	
≤0.9	71 (25.8%)	13 (18.1%)	34 (29.3%)	24 (27.6%)	0.126
<0.4	30 (10.9%)	7 (9.7%)	9 (7.8%)	14 (16.1%)	

Sufficiency, serum 25-OH-vitamin D ≥50 nmol/L; Insufficiency, 30 nmol/L ≤ 25-OH-vitamin D <50 nmol/L; Deficiency, serum 25-OH-vitamin D <30 nmol/L. 25-(OH)-VD, serum 25-OH-vitamin D; ABI, Ankle Brachial Index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rat. Values in bold represent variables with statistically differences.

survival comparisons were carried out for different grouping of vitamin D categories (six of these cases were deleted due to an uncertain time of death). Patients with vitamin D deficiency (<30 nmol/L) had significantly higher all-cause mortality than those without vitamin D deficiency (HR 0.571, 95% CI 0.329–0.991, P = 0.03; Figure 1a). However, patients with sufficient

vitamin D had a lower all-cause mortality than those with insufficient vitamin D, but the difference was not significant (Figure 1b,c).

Furthermore, the serum vitamin D concentrations were treated as both categorical variables and continuous variables for Cox regression analysis. In the multivariate models, we adjusted for age (≤55 years or >55 years) and sex (male or female) in model 1. Because vitamin D concentrations are affected by factors, such as sunlight, latitude and lifestyle, and vitamin D intake is vulnerable to confounding by socioeconomic factors, such as education[19]. Taking this into account, in model 2, we further adjusted for body mass index (kg/m^2), education level (less than high school, high school or equal, or college or above), residence (urban, villages, or country), smoking status (smoking or non-smoking),and Wagner grades (Wagner 1–3 or Wagner 4–5). In model 3, we further adjusted for the duration of DFU (months), glycated hemoglobin (%) and low-density lipoprotein (mmol/L). As shown in Table 2, vitamin D deficiency was significantly associated with higher all-cause mortality before (HR 0.570, 95% CI 0.341–0.954, P = 0.032) and after completely adjusted (HR 0.565, 95% CI 0.338–0.946, P = 0.030 in model 3). The result was similar when serum vitamin D concentrations were treated as continuous variables, and after completely adjusted, per 1-nmol/L decrease in the serum vitamin D concentration was associated with a 2.1% increased risk of all-cause mortality (Table 3).

Wound healing

For wound healing, we respectively assessed the 12-week healing rate, total healing rate, healing time and cumulative healing rate. Wounds healed by 12 weeks in 124 out of 200 patients with DFU, with a 12-week healing rate of 62.00%. There was no significant difference in vitamin D levels between the healed and unhealed group during 12 weeks (38.58 [IQR 28.24–52.65] vs 36.73 [IQR 27.32–51.08] nmol/L, P = 0.334). At the end of follow up, 167 out of 213 patients had healed ulcers with a total healing rate of 78.40%. The vitamin D concentrations in patients with unhealed ulcers was lower than that of patients with healed ulcers, but the difference was not significant (34.19 [IQR 26.80–50.58] vs 38.97 [IQR 28.25–52.14] nmol/L, P = 0.19). As for the healing time, 155 patients were evaluated who were divided into two groups according to vitamin D status (43 patients in the vitamin D deficiency group [<30 nmol/L] and 112 patients in the vitamin D non-deficiency group [≥30 nmol/L]). The healing time of the vitamin D deficiency group (51 days [IQR 23–74 days]) was longer than that of the vitamin D non-deficiency group (41 days [IQR 17–81 days]), but the difference was not statistically significant (P = 0.382). Finally, we carried out three comparisons of cumulative healing rates by different groupings based on vitamin D status. We did not find differences in cumulative healing rates among different vitamin D statuses (Figure 2).

Recurrence

Among 179 effective follow-up cases, 60 recurrences recurred, with a recurrence rate of 33.52%. There was no significant difference in serum vitamin D concentrations between the two groups (39.46 [IQR 29.81–52.62] vs 36.60 [IQR 27.13–48.54] nmol/L, P = 0.188). The Kaplan–Meier curves of vitamin D status and wound recurrence rates were shown in Figure 3. There was no significant difference in the cumulative recurrence rate among different vitamin D statuses.

Amputation

Furthermore, we evaluated the effect of vitamin D levels on amputation in patients with DFUs. According to the amputation status, the patients were divided into amputation group (n = 33) and non-amputation (n = 173) group. The amputation rate was 16.02%. The concentration of serum vitamin D in the amputation group was lower than that in the non-amputation group, but the difference was not statistically significant (34.00 [IQR 26.90–41.81] vs 40.21 [IQR 29.60–

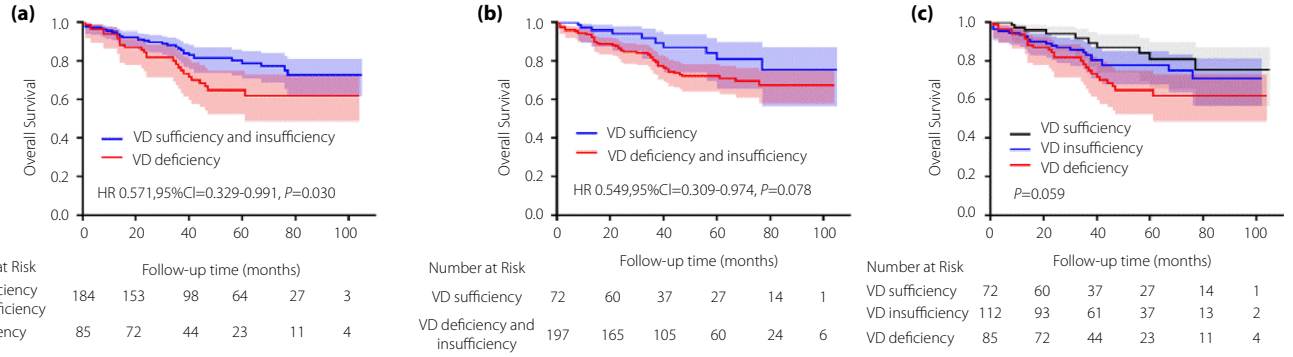

Figure 1 | The Kaplan–Meier curves show that patients with vitamin D (VD) deficiency had significantly higher all-cause mortality than those without vitamin D deficiency (hazard ratio [HR] 0.571, 95% confidence interval [CI] 0.329–0.991, P = 0.03), whereas there were no significant differences between patients with sufficient and insufficient vitamin D.

Table 2 | Hazard ratios of all-cause mortality by different categories of 25-OH-vitamin D among patients with diabetic foot ulcers

	Total (n = 269)	Vitamin D status		P
		Vitamin D deficiency (n = 85) HR (95% CI)	Vitamin D non-deficiency (n = 184) HR (95% CI)	
All-cause mortality (n/%)	59 (21.9%)	26 (30.6%)	33 (17.9%)	
Unadjusted model		1	0.570 (0.341, 0.954)	0.032
Model 1†		1	0.570 (0.341, 0.954)	0.032
Model 2‡		1	0.579 (0.346, 0.968)	0.037
Model 3§		1	0.565 (0.338, 0.946)	0.030

†Model 1: adjusted for age (≤55 years or >55 years) and sex (male or female). ‡Model 2: further adjusted (from model 1) for body mass index (kg/m^2), education level (less than high school, high school or equal, or college or above), residence (urban, villages or country), smoking status (smoking or non-smoking) and Wagner grades (Wagner 1–3 or Wagner 4–5). §Model 3: further adjusted (from model 2) for duration of diabetic foot ulcer (months), glycated hemoglobin (%) and low-density lipoprotein (mmol/L).
CI, confidence interval; HR, hazard ratio.

Table 3 | Hazard ratios of all-cause mortality by different levels of 25-OH-vitamin D among patients with diabetic foot ulcer

	Total (n = 269)	Vitamin D concentrations (nmol/L)		
		HR	95% CI	P
All-cause mortality (n/%)	59 (21.9%)			
Unadjusted model		0.978	0.962, 0.995	0.009
Model 1†		0.979	0.962, 0.995	0.013
Model 2‡		0.979	0.963, 0.996	0.014
Model 3§		0.979	0.963, 0.995	0.013

†Model 1: adjusted for age (≤55 years or >55 years) and sex (male or female). ‡Model 2: further adjusted (from Model 1) for body mass index (kg/m^2), education level (less than high school, high school or equal, or college or above), residence (urban, villages, or country), smoking status (smoking or non-smoking) and Wagner grades (Wagner 1–3 or Wagner 4–5). §Model 3: further adjusted (from Model 2) for duration of diabetic foot ulcer (months), glycated hemoglobin (%) and low-density lipoprotein (mmol/L). CI, confidence interval; HR, hazard ratio.

53.96] nmol/L, P = 0.053). In addition, we also compared the effect of vitamin D status on cumulative amputation rates and the Kaplan–Meier curves are shown in Figure 4. There was no significant difference in cumulative amputation rates among different vitamin D statuses.

DISCUSSION

Evidence from observational studies have previously shown an inverse association between vitamin D concentrations and all-cause mortality[19,20] and cause-specific mortality, such as cancer and cardiovascular disease[20-22]. Similar observations have been reported in the diabetes population[23-28]. Our previous study[13] showed a significant negative correlation between vitamin D concentrations and the prevalence of DFUs among Chinese type 2 diabetes mellitus patients.

In the present study, patients with DFUs who died at follow up had lower serum vitamin D concentrations than those who were alive. The finding that vitamin D deficiency was significantly associated with increased all-cause mortality in patients

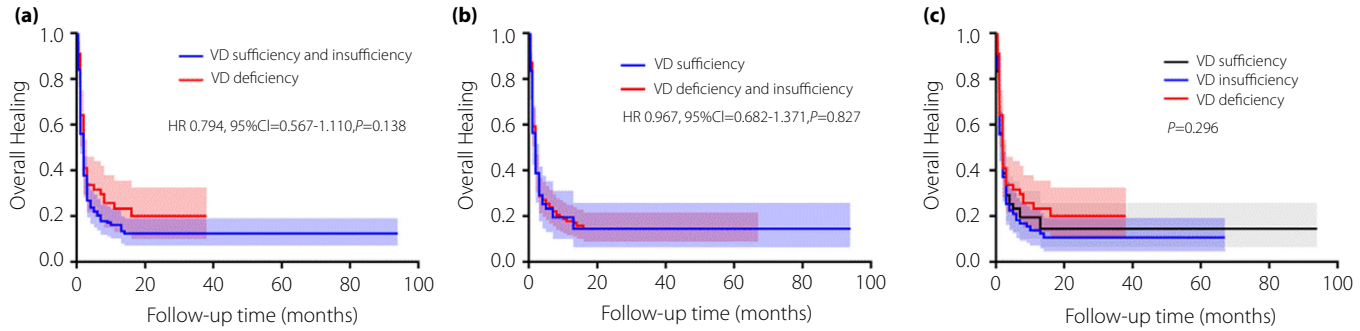

Figure 2 | The Kaplan–Meier curves show there were no significant differences in cumulative healing rates among different vitamin D (VD) statuses. CI, confidence interval; HR, hazard ratio.

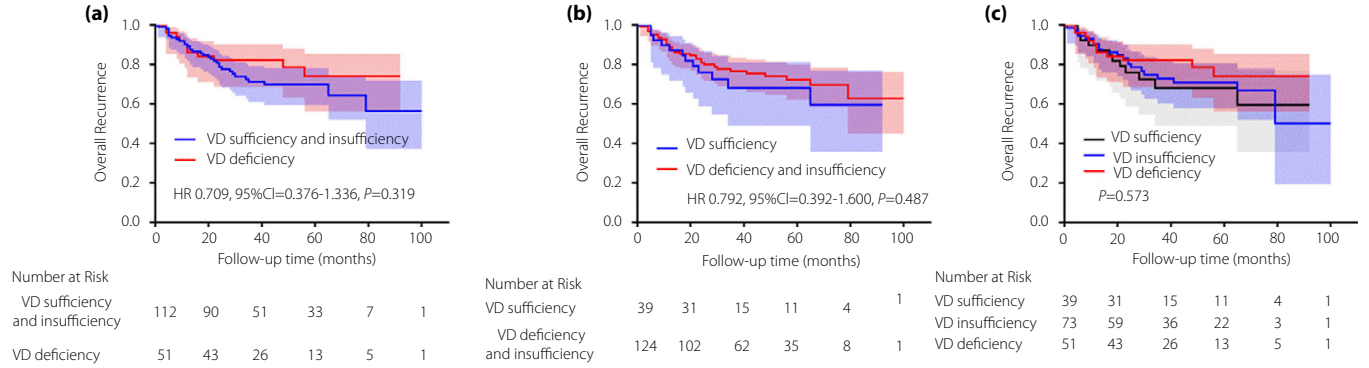

Figure 3 | The Kaplan–Meier curves show there were no significant differences in cumulative recurrence rates among different vitamin D (VD) statuses.

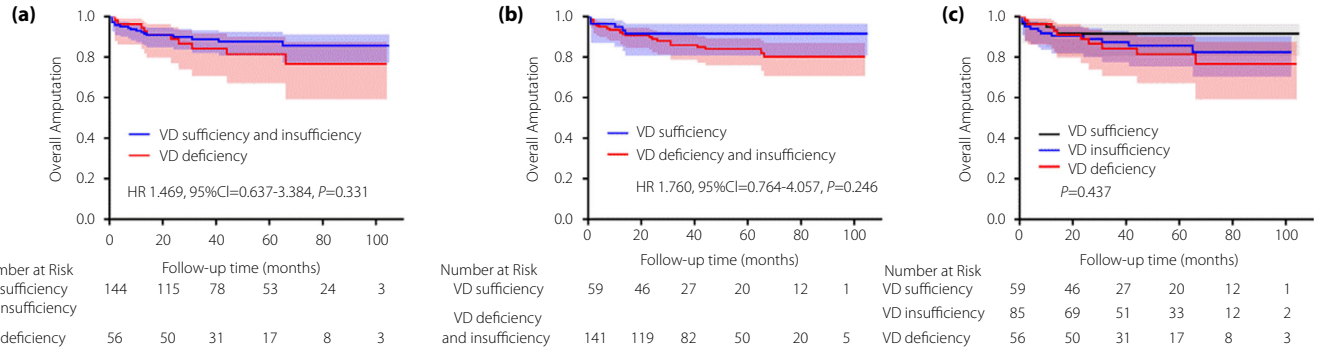

Figure 4 | The Kaplan–Meier curves show there were no significant differences in cumulative amputation rates among different vitamin D (VD) statuses.

with DFU in the present study was consistent with previous studies in the general population and diabetes population. This association was independent of traditional risk factors, including age, sex, body mass index, smoking status, Wagner grades and duration of DFUs, whether vitamin D was treated as a continuous variable or a categorical variable. This correlation persisted even after further adjusting for covariates, including education level, residence, glycated hemoglobin and low-density lipoprotein. In 2010, an observational study by Joergensen *et al.*[25] of 289 patients with type 2 diabetes mellitus who were followed for a median follow-up time was 15.0 years (IQR 0.2–23) first showed that severe vitamin D deficiency predicted an increased risk of all-cause mortality. They achieved a similar conclusion for people with type 1 diabetes[26]. Subsequently, growing evidence from observational studies with relatively consistent results has pointed to the possibility that vitamin D concentrations are inversely associated with all-cause mortality in diabetes populations[23,24,27,28], with possible sex differences[27]. Few data are currently available regarding diabetes-related complications[26], especially DFUs. To the best of our knowledge, this is the first report of an inverse association between vitamin D concentrations and all-cause mortality in patients with DFUs. However, despite observational evidence showing that, besides DFU, vitamin D deficiency has been linked to increased mortality[20-22] and various other diseases[29], including cancer, cardiovascular disease, respiratory infections and autoimmune diseases, vitamin D supplementation in randomized clinical trials (RCTs) and Mendelian randomization studies provided inconsistent results[29]. One possible reason is that observational studies are susceptible to uncontrolled confounding. Lower rates of severe vitamin D deficiency in the populations recruited in most RCTs and Mendelian randomization studies remain possible, as vitamin D might only work in patients with long-term and very severe vitamin D deficiency. Therefore, the causal relationship between vitamin D and the mortality of DFUs or other diseases is not clear. Further research is required to examine whether vitamin D supplementation contributes to an increased lifespan and improved prognosis.

We further assessed the outcomes of DFU, including the incidence of wound healing, recurrence and amputation. The positive effects of vitamin D in accelerating wound healing have shown promise in many preclinical studies. For example,

vitamin D could reduce the persistent inflammation through suppressing nuclear factor-κB-mediated inflammatory gene expression[31], and modulate innate immunity by inducing antimicrobial peptide gene expression through Toll-like receptor signaling[32]. Vitamin D receptor is required for re-epithelialization of wounds, as well as proliferation, migration and differentiation of epidermal stem cells[33]. Furthermore, it could promote angiogenesis through enhancing the expression of pro-angiogenic factors, including vascular endothelial growth factor A, hypoxia-inducible factor-1α and angiogenin[34]. However, the present results failed to show the difference in the levels of vitamin D between patients who experienced a healed wound during the follow up and those who still had an unhealed wound. It was also true for the assessment of the 12-week healing rate, healing time and cumulative healing rate. Actually, evidence from RCTs was reported by Razzaghi *et al.*[35] as early as 2017. The results showed that after 12 weeks of vitamin D supplementation in the experimental group of 30 participants, the parameters of wound healing were significantly improved compared with the placebo. Since then, similar results were also reported in two RCTs[36,37] with small sample sizes. The possible reason for the involvement of vitamin D supplementation in accelerating wound healing in DFUs is that it might lead to an improvement in glycemic control[6,38], as well as diabetic peripheral neuropathy[39,40] and peripheral arterial disease[10,11]. However, all of the aforementioned three RCTs, which could be currently available, included a relatively small sample size and did not assess potential confounders, such as physical activity, sunlight time and dietary factors, which could have an impact on vitamin D levels. The reason why we did not observe significant differences in the present study might be that our study had a relatively high loss to follow-up rate and only one-time point assessment of vitamin D. Therefore, additional studies are necessary to provide evidence supporting the role of vitamin D in wound healing and DFUs.

Although recurrence rates of DFUs are high and vary widely in different regions, the global recurrence rate of DFUs of 22.1% per person-year was calculated in a recent meta-analysis[41]. In the present study, the overall recurrence rate was 33.52%, which might be impacted on account of a large variation in the follow-up period and a relatively high rate of loss to follow up among participants. No significant differences were observed in vitamin D concentrations between different recurrence status, nor for cumulative recurrence rates. As for amputation, we calculated the incidence of amputation in DFUs patients was 16.02%, which was close to a previous report in China[42]. It is worth noting that, although not significant, we found vitamin D concentrations in the amputation group were lower than the non-amputation group, with a *P*-value very close to 0.05 ($P = 0.053$). The associations of vitamin D with amputation were likely to be underestimated for several reasons, such as a relatively small sample size and a one-time point assessment of vitamin D. Future studies with larger sample sizes might be able to confirm this relationship.

As vitamin D insufficiency or deficiency led to increased adverse events in patients with DFUs, especially all-cause mortality, accordingly, we propose the following suggestions targeting the management of DFU. First, routine measurement of serum vitamin D concentrations is deemed necessary in individuals with DFUs, irrespective of geographic locations and seasons of onset. Second, vitamin D supplementation of patients with insufficiency or deficiency is advised. Specifically, for individuals with normal hepatic and renal function, the most common forms of vitamin D supplements are cholecalciferol and ergocalciferol[42] due to their lower cost, and cholecalciferol is more efficient than ergocalciferol[43]. Otherwise, patients should be treated with hydroxylated vitamin D metabolites (e.g., calcifediol or calcitriol) when there is abnormal liver or kidney function[44,45]. Third, periodic monitoring is desirable during vitamin D supplementation to determine the proper dose, because either a deficiency[46] or an excess[47,48] of vitamin D is unfavorable. Fourth, moderate exposure to direct sunlight is required for patients with DFUs in addition to general precautions.

However, there has currently been a growing body of studies on the assessment of risk factors for the prognosis of DFUs[49-51], some of which also focus on the impact of nutritional status[52-54]. However, no studies to date have systematically assessed the relationship of vitamin D and the outcomes of DFUs. In this present study, vitamin D deficiency was associated with increased all-cause mortality in DFU patients. The positive role of vitamin D in wound healing is supported by both preclinical and clinical evidence. Thus, vitamin D supplementation is a promising potential adjunctive therapy for DFUs.

Globally, in 2015, the diabetes expenditure was estimated to be $1.3 trillion, up to one-third of which was spent on lower limb-related problems in the USA. In the UK, the total annual cost of management of DFUs was estimated to exceed $1.32 billion[56]. Recently, a single-center retrospective review in China showed that the total cost of DFUs management per patient was $6,217.80 in 2020, with an average of $3,228.20, and it has tended to increase year by year[57]. Thus, if adequate vitamin D concentrations were beneficial to the improvement of the state of wound healing, vitamin D supplementation, as a potential therapeutic for DFUs, would be a safe, economical, and widely available method to improve prognosis and reduce mortality among individuals with DFU. However, the association of vitamin D status with the prognosis of DFUs deserves further investigation, which might have important clinical implications.

One element of methodological strength was that the present study was a cohort study rather than a cross-sectional study, which provided an accurate and comprehensive analysis, and had a relatively large sample size. In addition, we adjusted for a multitude of sociological factors to minimize confounding. There were some limitations to the present study, beyond those that were inherent to retrospective research. The serum

vitamin D concentrations were tested only once on admission. In addition, for the determination of the serum vitamin D concentrations, the electrochemiluminescence immunoassay tended to provide an inaccurate assessment of 25-OH-vitamin D_2[58], thereby underestimating the total 25-OH-vitamin D. Despite controversy, liquid chromatography with tandem mass spectrometry has been currently served as the gold standard for measuring total 25-OH-vitamin-D in the circulation[59]. Third, the study population included were all inpatients and, therefore, this would be biased to more serious ulcers rather than ambulatory care-treated lesions. In addition, our assessment for the severity of ulcers was inadequate, because we used Wagner classification, which was obtained from the electronic medical record system. However, the International Working Group on the Diabetic Foot currently recommend the use of Site, Ischemia, Neuropathy, Bacterial Infection Area and Depth (SINBAD) system for the evaluation of DFUs. In fact, it would be difficult for us to retrospectively obtain those parameters for SINBAD scoring, such as area, depth, site on the foot, presence of infection and ischemia. Fourth, although additional follow up provided highly valuable clinical data, centralized telephone follow up was carried out only once, which led to incomplete information and recurrence might be ignored in patients with neuropathy. In addition, some patients were unwilling to be visited or could not be reached by telephone follow up were also reasons for such a high rate of loss to follow up, especially for deceased patients. Thus, such withdraw bias might lead to offset results, and the effects of vitamin D on DFU might be underestimated. Further limitations of the current study were connected with possible changes in vitamin D concentrations. We did not adjust for seasonal change, physical activity, sunlight time and dietary factors, which were associated with vitamin D levels. We also did not access the cause of death.

However, more observational studies are required to confirm this finding, and large RCTs are required to elucidate the role of vitamin D supplementation as a prophylaxis or treatment for DFUs.

Vitamin D deficiency was significantly associated with increased all-cause mortality in Chinese type 2 diabetes mellitus patients with DFUs, whereas the relationship between vitamin D and other poor prognosis of the DFUs requires further study. Vitamin D supplementation, as a potential therapeutic for DFUs, has possible benefits in the postponement of premature death and the improvement of outcomes among individuals with DFUs.

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DISCLOSURE

The authors declare no conflict of interest.

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