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Correlation of vitamin D and diabetic peripheral neuropathic pain: A cross-sectional study

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Abstract

Objective: Although ample evidence exists on the association between vitamin D deficiency and diabetic peripheral neuropathy, only a few studies have distinguished between painful and painless forms. This study compared serum vitamin D levels in type 2 diabetes patients with and without painful neuropathy.

Methods: We enrolled 46 patients with diabetes (painless diabetic peripheral neuropathy, n = 15; painful diabetic peripheral neuropathy, n = 16; non-neuropathy, n = 15) and 15 healthy controls. Assessments included neurologic exams, Michigan Neuropathy Screening Instrument, electrophysiology, skin biopsy, and serum 25(OH)D measurements.

Results: The level of 25(OH)D was significantly lower in painful diabetic peripheral neuropathy (23.00 ± 7.97) patients versus controls (44.48 ± 6.77), non- diabetic peripheral neuropathy (31.22 ± 6.14) patients, and painless diabetic peripheral neuropathy patients (36.91 ± 6.22; $P < 0.01$). Multivariate analysis identified vitamin D as an independent factor for painful diabetic peripheral neuropathy ($P = 0.007$). The 25(OH)D level was significantly correlated with sural amplitude ($r = 0.377$), peroneal amplitude ($r = 0.434$), vibration threshold ($r = -0.393$), and Michigan Neuropathy Screening Instrument score ($r = -0.456$).

Conclusions: Serum 25(OH)D levels were significantly lower in painful diabetic peripheral neuropathy, suggesting the potential role of vitamin D deficiency in the pathogenesis of diabetic peripheral neuropathy.

Keywords

Vitamin D, pain, diabetic peripheral neuropathy, type 2 diabetes, cross-sectional study

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Introduction

Diabetes is a pervasive noncommunicable disease, with projections indicating that over 1.31 billion people worldwide will be affected by 2050.[1] Diabetic peripheral neuropathy (DPN) is among the most frequent complications of type 2 diabetes mellitus (T2DM). Painful DPN (p-DPN), characterized by distressing symptoms such as tingling, burning, and electric shock-like pains in the distal extremities, affects 15%–25% of individuals with diabetes, with a higher prevalence in those with T2DM.[2,3] These painful symptoms severely impair patients' quality of life, impacting sleep, mobility, emotional well-being, and social functioning. Furthermore, p-DPN is a major contributor to disability, leading to foot ulcers, amputations, and fall-related injuries.[4–6] The underlying mechanisms of p-DPN remain incompletely understood. Although current clinical guidelines support the use of several pharmacological agents (e.g. gabapentinoids, serotonin-norepinephrine reuptake inhibitors, and tricyclic antidepressants), many patients achieve only suboptimal pain relief or experience dose-limiting adverse effects. This therapeutic gap underscores the need to identify novel modifiable risk factors and therapeutic targets.

Vitamin D deficiency is a global health issue and is considered a potential pandemic afflicting more than a billion people worldwide.[7] Recent years have witnessed increasing research interest in the association between vitamin D and DPN. Vitamin D deficiency is prevalent across various forms of diabetes, including prediabetes, type 1 diabetes, T2DM, and gestational diabetes, and may contribute to an increased risk of the disease.[8–12] Accumulating evidence further suggests that vitamin D deficiency serves as an independent risk factor for the development of DPN,[13] especially p-DPN.[14–16] In addition, vitamin D supplementation has shown potential benefits in alleviating symptoms of painful DPN.[17] The precise mechanisms linking vitamin D levels to DPN are not fully elucidated. Pain sensitivity associated with low vitamin D levels may be directly mediated by its action on dorsal root ganglia and small nerve fibers.[18] Studies have demonstrated greater small nerve fiber damage in patients with p-DPN than in those with painless diabetic neuropathy.[19] Conversely, vitamin D deficiency has also been implicated in increasing the risk of DPN in older patients with T2DM by exacerbating large-fiber lesions.[20] Although existing studies provide some evidence regarding vitamin D's role in DPN prevention and treatment, they have notable limitations. Most studies have failed to distinguish p-DPN from painless DPN and sample sizes have often been small, limiting statistical power and generalizability. Therefore, more robust and convincing data are warranted.

We therefore conducted this cross-sectional study to compare serum vitamin D levels, neurophysiological parameters, and intraepidermal nerve fiber density (IENFD) between patients with p-DPN and those with painless DPN. We hypothesized that lower serum vitamin D levels are associated with painful DPN and that this association is independent of IENFD, suggesting a potential role of vitamin D in pain modulation beyond small-fiber loss.

Materials and methods

Study design

This cross-sectional study included 46 patients with T2DM classified into 3 groups: (a) painless DPN (n = 15); (b) p-DPN (n = 16); and (c) diabetes without neuropathy (n = 15). These patients were then compared with 15 healthy controls matched for age and sex. This study was conducted at the Nanjing Gaochun People's Hospital between March and June 2022 and aimed to evaluate the association between serum 25-hydroxyvitamin D levels and DPN. Vitamin D status was assessed using chemiluminescence immunoassay, and DPN was diagnosed using the Douleur Neuropathique-4 (DN4) questionnaire and clinical assessment.

Setting

This cross-sectional study was conducted at the Metabolic Management Center of Nanjing Gaochun People's Hospital, China. Consecutive patients attending the routine diabetes follow-up clinic from March to June 2022 were assessed for eligibility. Recruitment occurred within this same period. All exposure (vitamin D measurement) and outcome (painful neuropathy assessment) data were collected at a single study visit for each participant. Thus, the data collection period coincided with the recruitment period.

Patient selection

Patients diagnosed with T2DM were recruited from Nanjing Gaochun People's Hospital, while healthy controls were enrolled from the hospital's physical examination center. Patients were recruited consecutively from the Metabolic Management Center between March and June 2022. All eligible patients during this period were invited to participate. T2DM was diagnosed according to the World Health Organization diagnostic criteria 1999 and the 2021 American

Diabetes Association criteria.[21,22] Exclusion criteria were patients with previous neurological disease (such as diseases affecting the central nervous system, known disorders of peripheral vascular, or hyperplasia or tumors derived from neuroendocrine cells), body mass index (BMI) less than 18 kg/m^2 or greater than 30 kg/m^2, severe renal dysfunction (estimated glomerular filtration rate (eGFR) <45 mL/min), severe liver dysfunction (aspartate or alanine aminotransferase level higher than three times the normal level), heart failure (New York Heart Association class III or IV), and cancer or infection within the past 3 months. To minimize selection bias, we used consecutive sampling, recruiting all eligible participants without arbitrary exclusion based on factors such as sunlight exposure duration or dietary intake. This study was conducted in accordance with the Declaration of Helsinki (2024 revision) of the World Medical Association. Ethical approval for the study was granted by both the hospital scientific and ethical committees on 21 November 2021 in Nanjing, Jiangsu Province, China (AF/SC-05/01.0). Informed consent was signed by all participants. Medical records were accessed for research purposes on 15 July 2022. Authors had access to identifiable information during data extraction from electronic health records; however, all data were de-identified prior to analysis. This study was reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[23]

Anthropometrics and other biochemical parameter measurement

BMI was calculated by the standard formula (weight (kg) / height (m^2)). The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured after 15 min of rest using a mercury sphygmomanometer. Following overnight fasting, blood samples were obtained from all participants through venipuncture. Fasting plasma glucose (FPG), triglycerides (TG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum creatinine (Scr) were measured using an automatic biochemistry analyzer (AU5800; Beckman Coulter, America). The endogenous creatinine clearance rate was calculated using the Cockcroft equation: GFR = [140 – age (years) * body weight (kg)] / [0.818 * serum creatinine (Scr, umol/L)] for males and the result *0.85 for females. Glycated hemoglobin (HbA1c) measurement was performed using high-performance liquid chromatography (HA-8180; ARKRAY, Japan). Urinary albumin concentration was measured by immunonephelometry (AU5800; Beckman Coulter, America). The urinary albumin-to-creatinine ratio (ACR) was determined as urine albumin (mg)/urine creatinine (g). All measurements were repeated twice.

Vitamin D assay

To minimize potential confounding from seasonal variations in sun exposure, serum vitamin D levels were measured exclusively during the spring months (March to June). We used an electrochemiluminescence binding assay for the quantitative determination of total serum 25(OH)D (Roche Diagnostics, USA). Blood samples were collected only during the spring months (March to June). Inter-assay variability was 6.5% and intra-assay variability was 3.1% at 28 ng/mL.

Neuropathy assessment

In our Metabolic Management Center, all participants underwent a thorough assessment with the neuropathy symptom profile. Those participating in the study underwent evaluation of the DN4 questionnaire proposed by Bouhassira et al. in France in 2005.[24] The DN4 scale is a simple tool comprising 10 questions, with a total score ≥4 points considered as neuropathic pain and that of <4 considered as non-neuropathic pain. The Michigan Neuropathy Screening Instrument (MNSI) score, a validated screening tool for DPN,[25] was simultaneously scored. Neurologic deficits, including unequivocally decreased or absent reflexes and decreased distal sensations for neuropathy, were confirmed through physical examination with the following tools using the modified neuropathy disability score: (a) 10-g monofilament for touch sensation test (four sites per foot); (b) pin for pain sensation test; (c) tendon hammer for reflexes test; and (d) Digital Vibration Threshold Tester for vibration sensation test. The indicators were recorded according to the neurological impairment rating: 0 indicating no impairment; 1 indicating slight impairment; 2 indicating moderate impairment; 3 indicating severe impairment; and 4 indicating a complete loss of function or the most severe impairment.[26] Electromyography was used to measure ulnar, median, sural, and peroneal nerve conduction velocities on the right side. If two or more nerves exhibited abnormal results, nerve conduction was deemed abnormal.[27] Patients with diabetes were grouped according to the following criteria: (a) p-DPN: MNSI >2 or abnormal nerve conduction and DN4 score ≥4; (b) painless DPN: MNSI >2 or abnormal nerve conduction and DN4 score <4; and (c) Diabetes without neuropathy: normal MNSI (≤2), normal nerve conduction, and DN4 score <4.

Immunohistochemistry and skin biopsy

IENFD was evaluated in participants who received a 3-mm punch skin biopsy from the left lateral calf, approximately 10 cm above the lateral malleolus after local anesthesia (2% lidocaine). The biopsy sample was promptly fixed in 4% paraformaldehyde for 24 h, immersed in 30% sucrose (<24 h) at 4°C, embedded in optimal cutting temperature-embedding compound, swiftly frozen in liquid nitrogen, and sliced into 50-μm sections utilizing a cryostat (CM1950; Leica, Germany). After washing in phosphate-buffered saline three times for 10 min each, floating sections were subjected to a protein block with a solution of 5% bovine serum albumin and 0.3% Triton X-100 at room temperature for 2 h. Subsequently, the sections were incubated in a PGP9.5 monoclonal antibody (GB12159, Servicebio, China) solution overnight at 4°C. The next day, the slides were washed three times for 10 min each in PBST containing 0.05% Tween 20, followed by 1.5-h incubation at room temperature in the dark with a secondary antibody. After rinsing the slides thrice for 10 min each in PBST in the dark, the tissues were cover-slipped with anti-fluorescence quenching medium containing 4′,6-diamidino-2-phenylindole (DAPI) and examined under a microscope. IENFD was performed in accordance with the following established criteria: 1. The number of nerve fibers entering the epidermal layer from the dermis through the basement membrane was one. 2.The IENFD(number/mm) was calculated by dividing the number of intraepidermal nerve fiber by the length of the epidermal layer.[28,29]

Statistical analysis

Categorical data were expressed as frequencies and percentages; chi-square tests were used. Continuous variables were presented as the mean and standard deviation (SD). The Kolmogorov–Smirnov test was used to assess the normality of continuous data. Furthermore, data from the normal distribution were examined using one-way analysis of variance with the implementation of Bonferroni's post-hoc analysis. The data that did not follow a normal distribution were assessed using the Kruskal–Wallis test and Bonferroni test. In addition, we used Spearman correlation tests to assess correlations between vitamin D and anthropometry and metabolic profiling. Logarithmic transformation was applied to non-normally distributed data prior to conducting correlation analysis. An analysis of covariance was performed to compare serum vitamin D levels between groups, with age, sex, BMI, HbA1c, diabetes duration, and eGFR included as covariates. Furthermore, the correlation between vitamin D and T2DM and DPN was examined via multiple logistic regression. The analyses were conducted using SPSS version 20 (SPSS, USA), with a significance threshold set at $p < 0.05$. Only variables that reached statistical significance in univariate analysis were included in the multivariate logistic regression model.

Results

Study participants

A total of 145 patients with T2DM attending the routine diabetes follow-up clinic at Nanjing Gaochun People's Hospital between March and June 2022 were assessed for eligibility. Of these, 44 did not meet the inclusion criteria, 45 declined to participate, and 10 had incomplete data (primarily missing vitamin D measurements or DN4 scores). Therefore, 46 eligible patients were included in the final analysis. These comprised 16 patients with p-DPN, 15 with painless DPN, and 15 with diabetes without neuropathy. In addition, 15 healthy controls matched for age and sex were enrolled for comparison.

Characteristics of study participants

There were no differences in the BMI, TC, LDL-C, age, and urinary ACR among the groups. In patients with T2DM, the fasting blood glucose (FBG), HbA1c, TG, SBP, and DBP were higher than those in controls, while HDL-C, GFR, and serum vitamin D level were lower (Table 1). Notably, the p-DPN group had a longer disease duration ($P = 0.042$) and significantly lower serum vitamin D levels ($P < 0.001$) than the painless DPN group (Table 1).

Clinical, neurophysiological, and skin biopsy data

The neuropathy groups exhibited significantly high MNSI scores and very low IENFD, indicating well-established DPN. The MNSI scores were significantly higher in p-DPN than in DPN ($P < 0.05$) and control participants ($P < 0.001$) (Table 2). Deep breathing-heart rate variability (DB-HRV) did not differ between the p-DPN and painless DPN groups (Table 2). Vibration perception threshold (VPT) was higher in patients with p-DPN than in those with DPN (Table 2). Although IENFD was significantly reduced in both neuropathy groups compared with the non-DPN group ($P < 0.01$), no statistically

Table 1. Clinical characteristics of all participants.

Variables	Healthy controls (n = 15)	Non-DPN (n = 15)	p-DPN (n = 16)	DPN (n = 15)	p-DPN vs. DPN			
					χ^2/F	P	t/Z	P
Age (years)	56.20 ± 7.13	57.07 ± 5.06	58.00 ± 6.29	58.53 ± 10.36	0.288	0.834	0.030	0.863
Male (%)	8 (53.3)	12 (80.0)	12 (75.0)	5 (33.3)	8.761	0.033		**0.032**
Smoking (%)	13 (86.7)	13 (86.7)	15 (93.8)	14 (93.3)	0.816	0.846		1.000
Duration (years)		7.28 ± 5.63	10.42 ± 8.69	5.01 ± 4.80	8.881	<0.001	4.517	**0.042**
BMI (kg/m^2)	21.94 ± 1.96	23.94 ± 3.76	23.83 ± 2.21	23.94 ± 3.42	1.71	0.175	0.012	0.913
FBG (mmol/L)	5.03 ± 0.46	13.60 ± 4.26	10.60 ± 4.73	11.19 ± 3.55	14.605	<0.001	0.151	0.700
HbA1c (%)	5.27 ± 0.19	10.30 ± 2.13	8.80 ± 2.36	9.09 ± 1.77	20.852	<0.001	0.151	0.700
TC (mmol/L)	4.21 ± 0.50	4.65 ± 0.89	4.88 ± 1.03	5.09 ± 1.55	1.885	0.142	0.186	0.669
HDL-C (mmol/L)	2.18 ± 0.99	1.18 ± 0.17	1.25 ± 0.27	1.34 ± 0.45	10.369	<0.001	0.435	0.515
LDL-C (mmol/L)	2.51 ± 0.45	2.80 ± 0.71	2.84 ± 0.74	2.96 ± 0.35	1.533	0.216	0.334	0.568
TG (mmol/L)	1.09 (0.81, 1.66)	1.17 (0.83, 1.56)	1.66 (1.13, 2.23)	1.90 (1.66, 2.67)	16.471	0.001	−1.285	0.202
SBP (mmHg)	120.80 ± 8.60	139.40 ± 15.06	133.19 ± 16.09	131.93 ± 19.86	3.769	0.015	0.038	0.848
DBP (mmHg)	75.07 ± 5.65	84.20 ± 10.78	81.81 ± 9.96	82.93 ± 9.77	2.854	0.045	0.097	0.758
eGFR (ml/min)	103.4 (101.4, 110.2)	103.6 (94.7, 112.6)	90.05 (51.78, 100.3)	96.5 (76.2, 98.7)	18.229	<0.001	−0.316	0.770
ACR (mg/g)	25.92 (12.10, 28.38)	6.18 (4.11, 46.20)	18.08 (8.52, 65.46)	20.68 (10.68, 33.56)	4.323	0.229	−0.119	0.922
Vitamin D (ng/mL)	44.48 ± 6.77	31.22 ± 6.14	23.00 ± 7.97	36.91 ± 6.22	27.248	<0.001	−5.389	<0.001

BMI: body mass index; FBG: fasting blood glucose; SBP: systolic blood pressure; DBP: diastolic blood pressure; TG: triglycerides; TC: total cholesterol; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; ACR: urine albumin-to-creatinine ratio; GFR, glomerular filtration rate; DPN: diabetic peripheral neuropathy; p-DPN: painful diabetic peripheral neuropathy.

Table 2. Neuropathy parameters.

Variables	Controls (n = 15)	Non-DPN (n = 15)	p-DPN (n = 16)	DPN (n = 15)	p-DPN vs. DPN			
					F/Z	P	t/Z	P
IENFD (fibers/mm)	9.73 ± 2.12	7.73 ± 1.83	4.81 ± 1.68	5.67 ± 1.76	21.678	<0.001	1.910	0.178
DB-HRV (beats/min)	20.20 ± 1.78	18.73 ± 1.39	17.38 ± 0.96	17.93 ± 1.49	11.322	<0.001	1.566	0.221
MNSI-Q		1.00 (0, 1.00)	7.00 (5.00, 8.00)	5.00 (4.00, 6.00)	31.544	<0.001	−2.294	**0.022**
MNSI-P		1.00 (1.00, 1.00)	4.25 (3.63, 5.00)	3.00 (2.50, 3.50)	36.871	<0.001	−3.776	**<0.001**
VPT (V)	10.24 ± 1.72	16.10 ± 3.36	25.71 ± 4.18	21.37 ± 3.74	59.605	<0.001	9.194	**0.005**
Sural amplitude (μV)	8.03 ± 1.58	7.67 ± 1.42	4.13 ± 1.25	6.03 ± 0.95	28.576	<0.001	22.522	**<0.001**
Sural SNCV (m/s)	48.33 ± 3.22	42.60 ± 4.85	33.06 ± 5.16	38.00 ± 3.72	34.952	<0.001	9.231	**0.005**
Peroneal MNAmp (μV)	5.60 ± 1.22	4.47 ± 1.16	1.87 ± 0.29	2.34 ± 0.56	59.745	<0.001	8.824	**0.006**
Peroneal MNCV (m/s)	45.47 ± 2.62	40.27 ± 3.97	34.63 ± 3.67	36.33 ± 4.32	25.918	<0.001	1.415	0.244

IENFD: intraepidermal nerve fiber density; DB-HRV: deep breathing-heart rate variability; MNSI: Michigan Neuropathy Screening Instrument; MNSI-Q: MNSI questionnaire score; MNSI-P: MNSI physical examination score; VPT: vibration perception threshold; sural SNCV: sural nerve conduction velocity; peroneal MNAmp: peroneal motor nerve amplitude; peroneal MNCV: peroneal motor nerve conduction velocity; DPN: diabetic peripheral neuropathy; p-DPN: painful diabetic peripheral neuropathy.

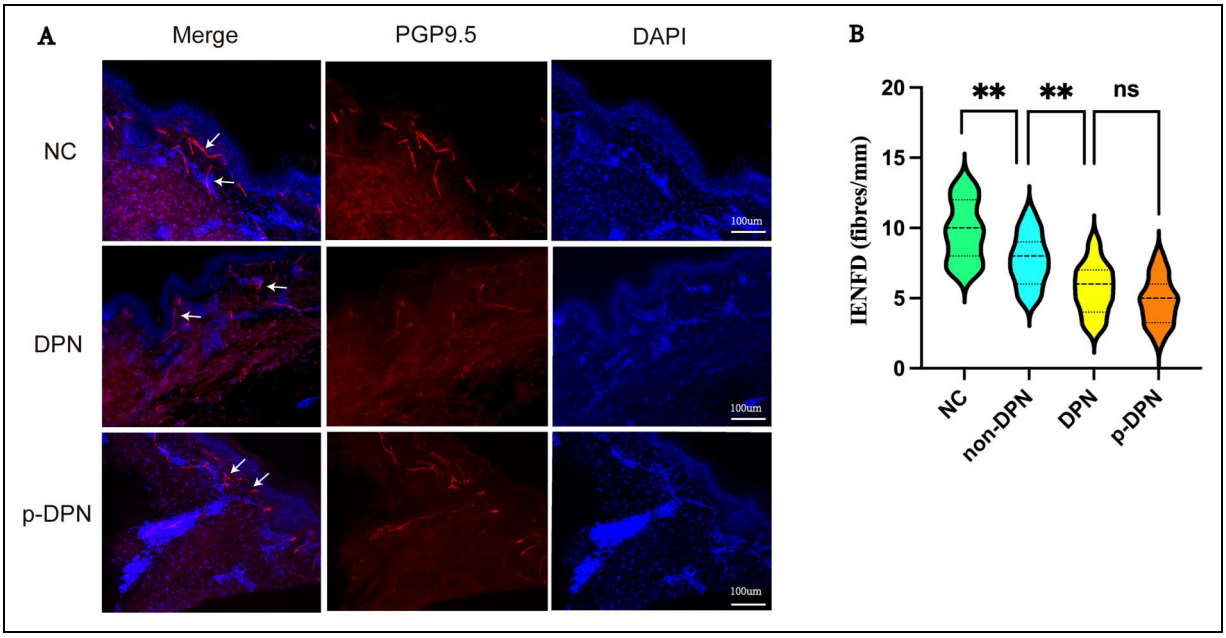

Figure 1. Small nerve fibers in the skin tissue. (a) Nerve fiber immunofluorescence staining with PGP9.5. (B) Analysis of intraepidermal nerve fiber density (IENFD) using Image J.
NC: normal control; DPN: diabetic peripheral neuropathy; p-DPN: painful diabetic peripheral neuropathy; PGP9.5: protein gene product 9.5. White arrows indicate the counted nerve fibers.

significant difference was observed in IENFD between the p-DPN and painless DPN groups ($P < 0.01$) (Table 2 and Figure 1).

Peroneal nerve conduction velocity and amplitude were significantly lower in DPN and p-DPN ($P < 0.001$) than in control participants, and the amplitude was lower in patients with p-DPN than in those with DPN ($P < 0.01$). Sural nerve conduction velocity and amplitude were significantly lower in DPN and p-DPN ($P < 0.001$) than in control participants and were lower in patients with p-DPN than in those with DPN ($P < 0.01$) (Table 2).

Serum vitamin D levels

Serum 25(OH)D levels were significantly lower across all diabetic groups than the healthy controls. Most notably, patients with p-DPN exhibited significantly lower 25(OH)D levels than those with painless DPN (23.00 ± 7.97 vs. 36.91 ± 6.22 ng/mL, $P < 0.001$) (Table 1 and Figure 2). A more nuanced analysis revealed that within the diabetic subgroups, interestingly and perhaps counterintuitively, serum 25(OH)D levels were higher in the painless DPN group than in the non-DPN group (36.91 ± 6.22 vs. 31.22 ± 6.14 ng/mL, $P < 0.05$).

Multiple regression analysis for 25(OH)D level and p-DPN

The univariate logistic regression analysis identified sex, disease duration, and serum 25(OH)D as potential factors associated with p-DPN. However, after adjusting for these variables in a multivariate logistic regression model, only serum 25(OH)D level remained independently associated with p-DPN (odds ratio (OR) = 0.800, 95% confidence interval (CI): 0.680–0.941, $P = 0.007$, Table 3). Neither sex nor diabetes duration reached statistical significance ($P > 0.05$ for both). In the univariate analysis, HbA1c did not reach statistical significance as a predictor of painful DPN ($p > 0.05$, Table 1) and was consequently not included in the multivariate regression model.

Receiver operating characteristic curve analysis was performed to evaluate the ability of serum vitamin D levels to differentiate patients with p-DPN from those with painless DPN. The results indicated that serum vitamin D level possessed excellent diagnostic value for identifying painful DPN, with an area under the curve (AUC) of 0.976 (Figure 3). The optimal serum 25(OH)D cutoff value for distinguishing painful DPN was determined to be 24.48 ng/mL, which yielded a sensitivity of 81.3% and a specificity of 93.3%.

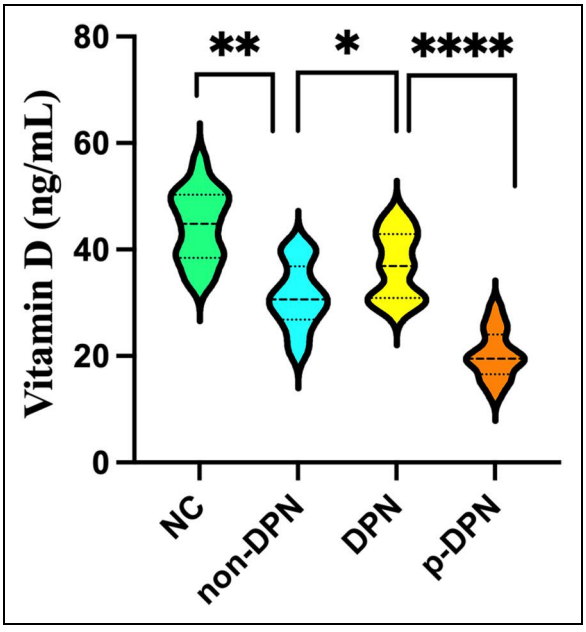

Figure 2. Serum vitamin D levels. The diabetes without neuropathy subgroup (non-DPN) showed significantly decreased serum 25(OH)D levels compared with the control group (NC). Serum 25(OH)D levels were significantly lower in diabetes with painful neuropathy (p-DPN) groups than in those with painless neuropathy (DPN).

Table 3. Logistic regression predicting the risk factors for painful DPN.

	B	SE	Wald	P	Odds ratio	95% CI
Male	−1.939	1.269	2.335	0.127	0.144	0.012–1.730
Duration	0.176	0.127	1.908	0.167	1.192	0.929–1.530
Vitamin D	−0.223	0.083	7.236	**0.007**	**0.800**	**0.680–0.941**
Constant	8.518	3.366	6.402	0.011	5001.635	

DPN: diabetic peripheral neuropathy; CI: confidence interval.

Correlation analyses were performed to explore the relationship between vitamin D levels and specific neuropathy parameters. Significant positive correlations were observed between serum 25(OH)D levels and sural nerve amplitude ($r = 0.377$, $P = 0.036$) as well as peroneal motor nerve amplitude ($r = 0.434$, $P = 0.015$) (Table 4). Conversely, significant negative correlations were found between 25(OH)D and VPT ($r = -0.393$, $P = 0.029$) and the MNSI Physical Examination Score ($r = -0.456$, $P = 0.01$) (Table 4). Although DB-HRV was reduced in neuropathy groups, it did not differ between p-DPN and painless DPN groups ($P = 0.221$, Table 2) and showed no correlation with the vitamin D levels ($r = -0.009$, $P = 0.96$, Table 4). Although IENFD was significantly lower in the combined DPN groups than in the non-DPN group and controls ($P < 0.001$; Table 2; Figure 1), no significant correlation was found between 25(OH)D level and IENFD ($r = 0.138$, $P = 0.46$, Table 4).

Discussion

Although p-DPN is a predominant cause of disability**,** contributing to foot ulcers, amputations, gait disturbances, and fall-related injuries, its underlying pathogenesis remains incompletely elucidated. Accumulating evidence from recent studies suggests an association between vitamin D deficiency and painful diabetic neuropathy.[13,14,30]

Our findings demonstrated significantly lower serum 25(OH)D levels in patients with p-DPN than in those with painless DPN patients, non-DPN patients, and healthy controls. Crucially, after adjusting for potential confounders through multivariate logistic regression analysis, serum 25(OH)D level remained independently associated with painful DPN, with excellent discriminative ability between painful and painless DPN (AUC = 0.976). Notably, this association was independent of IENFD, suggesting that vitamin D may influence pain through mechanisms beyond simple axonal degeneration.

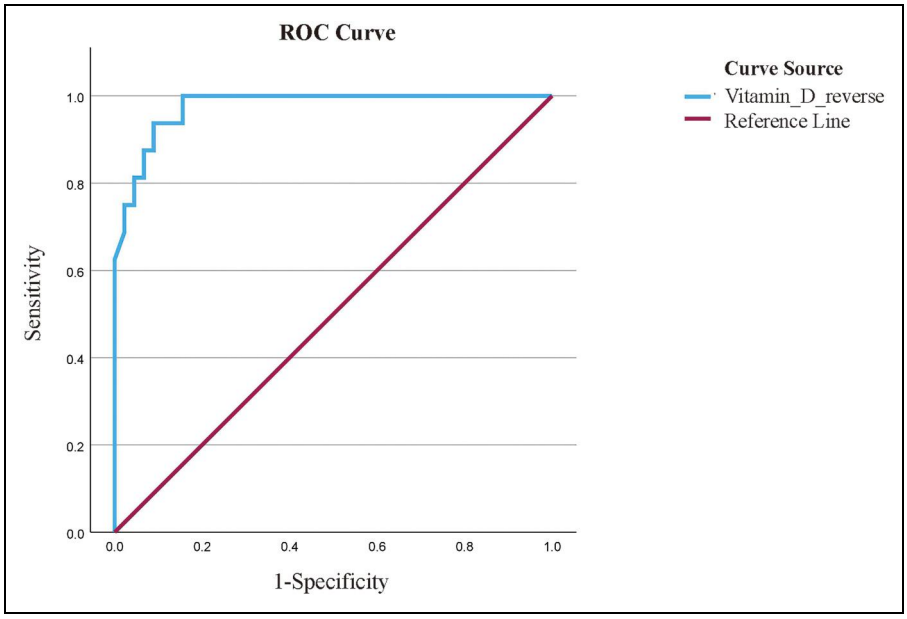

Figure 3. The receiver operating characteristic (ROC) curve for predicting painful DPN. The area under the ROC curve for vitamin D was 0.976. A vitamin D level of 24.48 ng/mL served as the optimal cutoff point, providing a sensitivity of 81.3% and a specificity of 93.3%.

Table 4. Correlation between vitamin D and neuropathy assessments in patients with neuropathy.

Variables	r	P
IENFD (fibers/mm)	0.138	0.460
DB-HRV (beats/min)	0.009	0.960
MNSI Questionnaire Score	−0.352	0.052
MNSI Physical Examination Score	−0.456	0.01
VPT (V)	−0.393	0.029
Sural amplitude (μV)	0.377	0.036
Sural SNCV (m/s)	0.111	0.551
Peroneal MNAmp (μV)	0.434	0.015
Peroneal MNCV (m/s)	0.254	0.168

IENFD: intraepidermal nerve fiber density; DB-HRV: deep breathing-heart rate variability; MNSI: Michigan Neuropathy Screening Instrument; MNSI-Q: MNSI questionnaire score; MNSI-P: MNSI physical examination score; VPT: vibration perception threshold; sural SNCV: sural nerve conduction velocity; peroneal MNAmp: peroneal motor nerve amplitude; peroneal MNCV: peroneal motor nerve conduction velocity.

An unexpected finding was that serum 25(OH)D levels were higher in the painless DPN group than in the non-DPN group. Several explanations are possible. First, residual confounding (e.g. differences in sun exposure, physical activity, or diet, which were not measured) may account for this observation. Second, reverse causation is possible: painless DPN does not impair mobility, allowing patients to maintain outdoor activity and vitamin D synthesis. Our cross-sectional design cannot distinguish between these possibilities, and longitudinal studies are warranted to clarify the underlying mechanisms.

Vitamin D deficiency increases the risk of DPN in older patients with T2DM by predominantly increasing large-fiber lesions.[20] Our results align with this notion, as we found significant correlations between serum 25(OH)D levels and parameters reflecting large-fiber function, including sural nerve amplitude, peroneal motor nerve amplitude, vibration perception threshold (VPT), and the MNSI physical examination score. This suggests that vitamin D deficiency may contribute to the severity of neuropathy, particularly affecting large fibers, in our cohort with well-established neuropathic disease. In contrast, although IENFD was significantly reduced in both neuropathy groups compared with the non-DPN group, we observed no significant difference in IENFD, a marker of small-fiber integrity, between the p-DPN and painless DPN groups. This finding appears to contrast with that of a study by Shillo et al.,[15] which proposed a role of vitamin D deficiency

specifically in small-fiber neuropathy, affecting nociceptors and correlating with sub-epidermal nerve fiber density but not affecting nerve conduction studies. The discrepancy with our results, wherein IENFD did not correlate with vitamin D levels, may be attributable to our relatively limited sample size and warrants further investigation in larger cohorts.

Several mechanisms may explain the link between vitamin D deficiency and painful DPN; however, our study did not directly test them. Vitamin D may act on sensory nerves directly, as calcitonin gene-related peptide (CGRP)-positive neurons show hormonally regulated vitamin D ligand and receptor levels.[31] It may also modulate pain signaling pathways such as nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF), and eGFR,[32] thereby exerting neuroprotective effects on peripheral nerves.[33] Additionally, vitamin D status may influence pain via gut microbiota alterations.[34] Important supportive evidence comes from studies of chemotherapy-induced peripheral neuropathy (CIPN), a condition pathophysiologically related to DPN. In both clinical studies and animal models, vitamin D deficiency is associated with more severe CIPN, and supplementation reduces pain behaviors and preserves nerve fiber density.[35,36] These parallels strengthen the biological plausibility that vitamin D deficiency contributes to pain in DPN through shared mechanisms (e.g. neuronal hyperexcitability, oxidative stress, and impaired neurotrophic support). Our cross-sectional findings cannot confirm any of these pathways; however, they provide a clinical rationale for future animal and cellular studies to investigate the causal mechanisms linking vitamin D deficiency to painful DPN.

There was a significant negative correlation between serum 25(OH)D levels and pain scores (DN4).[15] The addition of oral vitamin D (5000 IU) once daily over 8 weeks to standard treatment (pregabalin, gabapentin, or amitriptyline) significantly improves pain and mood in patients with diabetic neuropathy.[37] Oral supplementation of vitamin D3 (50,000 IU) once weekly for 12 weeks was associated with significant decrease in the symptoms and signs of diabetic neuropathy.[17] Treatment with a single intramuscular dose of high-dose vitamin D 600,000 IU of vitamin D in patients with painful diabetic neuropathy is associated with a significant decrease in the symptoms of painful diabetic neuropathy.[38] Although these findings are promising, our present cross-sectional study did not assess the therapeutic efficacy of vitamin D supplementation. Prospective, randomized controlled trials are needed to definitively establish its role in managing painful DPN.

Several limitations of this study should be acknowledged. First, its cross-sectional design precludes the establishment of a causal relationship between vitamin D deficiency and the development of painful DPN. Second, this study did not include a formal a priori sample size calculation. The sample size was based on consecutive recruitment over a fixed period (March–June 2022), the sample size was relatively small, which may limit the generalizability of the findings and the power to detect more subtle associations, particularly concerning small-fiber parameters. Findings should be considered exploratory, and future studies with larger, longitudinal cohorts are warranted to confirm and extend our observations.

In conclusion, this cross-sectional study found that lower serum vitamin D levels are associated with p-DPN and demonstrated excellent discriminative ability between p-DPN and painless DPN. However, given the observational design and modest sample size, these findings are hypothesis-generating only. They do not establish causality and should not be interpreted as supporting routine vitamin D screening or supplementation in clinical practice. Future prospective cohort studies and randomized controlled trials are needed to determine whether vitamin D plays a causal role in the pathogenesis of painful DPN and whether supplementation has any therapeutic or preventive value.

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Ethics approval and consent to participate

The protocols of the study were approved by the Ethics Committee of Gaochun Hospital Affiliated to Jiangsu University under the ethics code: AF/SC-05/01.0. Informed consent was obtained from all participants. This study follows the Declaration of Helsinki.

Author contributions

Caixia Yao: conceptualization, methodology, writing – original draft, supervision, project administration, and funding acquisition; **Shoucheng Zhang**: formal analysis, resources, validation, and writing – review & editing; **Li Wang:** data curation, formal analysis, and software; **Hongman Zhang:** data curation and visualization; **Wenping Yang:** validation and software; **Ying Zhong** and **Wenli Han:** investigation; **Ji Xu, Yan Chen** and **Ping Wang:** data curation.

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Declaration of conflicting interests

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data availability statement

The authors have full control of all data associated with this study, which are available for verification upon reasonable request.

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