Original Article

Relationship between vitamin D status and cardiac autonomic neuropathy in prediabetes

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Background and Objectives: Studies have suggested that vitamin D deficiency is associated with impaired cardiac autonomic nerve function. This study explores the correlation between serum vitamin D and cardiac autonomic neuropathy (CAN) in prediabetes and reveals the protective effect of vitamin D against CAN. **Methods and Study Design:** In total, 113 patients with prediabetes and definite CAN and 180 with prediabetes but without CAN were enrolled on the basis of their standard cardiovascular autonomic reflex test results. Chemiluminescence was used to measure 25(OH)D, the patients with CAN were divided into four groups, and the heart rate variability (HRV) of the study groups were compared. **Results:** Relative to the 50≤25(OH)D<75-nmol/L group, the 25≤25(OH)D<50-nmol/L and 25(OH)D<25-nmol/L groups exhibited significant differences in the time and frequency domains of HRV ($p<0.05$). Furthermore, we discovered that 25(OH)D is positively correlated with standard deviation of normal-to-normal RR intervals (SDNN) (β=0.566, $p<0.05$) and negatively correlated with low-to-high frequency ratio (LF/HF) (β=−0.199, $p<0.05$). A logistic regression reveals that CAN in prediabetes is significantly correlated with the 25(OH)D concentrations of <25 nmol/L (OR, 2.380 [1.208–4.691]; $p<0.05$) and 25≤25(OH)D<50 nmol/L (OR, 1.875 [1.064–3.751]; $p<0.05$). **Conclusions:** Serum 25(OH)D is significantly correlated with CAN in prediabetes, especially in the 25(OH)D <25-nmol/L group. Therefore, vitamin D deficiency may be related to the occurrence of CAN in prediabetes, and appropriate supplementation may provide protection against CAN.

Key Words: prediabetes, 25(OH)D, cardiac autonomic neuropathy, heart rate variability, diabetes

INTRODUCTION

Prediabetes is the condition that precedes diabetes. An epidemiological survey conducted by the International Diabetes Federation (2021) revealed that adult patients with impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) account for 10.6% and 6.2%, respectively, of the global population, and by 2045, these figures are expected to reach 11.4% and 6.9%, respectively. The pathogenesis of prediabetes is complex, and a series of complications can develop when the disease progresses. An increase in diabetes complications was previously believed to be positively correlated with the progression of diabetes; however, studies have revealed that patients with prediabetes may also sustain concomitant damage to terminal organs (e.g., the kidneys, retinas, nerves, blood vessels, and heart),[1] and CAN is a result of such damage.

The prevalence of CAN is 16.8% and 34.3% among individuals with type 1 diabetes and type 2 diabetes, respectively,[2] and it can be regarded as an independent predictor of CVD in patients with diabetes. However, studies have reported that even in the early stage of impaired glucose homeostasis, peripheral and autonomic small nerve fibres are damaged.[3] Autonomic neuropathy can lead to the impairment of various systemic functions, and the main manifestations of cardiac involvement include resting tachycardia, postural hypotension, exercise intolerance, intraoperative complications, and diabetic cardiomyopathy. In addition, impaired cardiac autonomic nerve homeostasis leads to abnormal norepinephrine signal transduction, resulting in dull pain or loss of pain sensation and increased asymptomatic myocardial ischemia and infarction.[4] These findings suggest that prediabetic CAN is associated with acute cardiovascular complications and significantly increases the risk of sudden cardiac death. Scholars have identified hypertension, smoking, hyperlipidaemia, obesity, and poor blood glucose control as risk factors for CAN.[5] In addition, studies have demonstrated that vitamin D deficiency is associated with cardiac autonomic nerve damage in individuals with prediabetes.

Vitamin D mainly regulates calcium homeostasis and bone metabolism, and it participates in immune inflammatory responses and cell proliferation, differentiation, and apoptosis. Moreover, vitamin D deficiency is associa-

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ted with an increased risk of CAN in patients with prediabetes. Vitamin D can affect progression from prediabetes to diabetes by promoting insulin secretion, improving insulin sensitivity, and increasing oxidase proliferation–activated receptor gene expression. Furthermore, vitamin D helps to protect peripheral nerve fibres by affecting the synthesis of neurotransmitters and reducing nerve demyelination and axon regeneration,[6] which can affect autonomic nerve function. Moreover, vitamin D acts on target organs (the heart, kidneys, and bone) through classic genomic translocations, and it is involved in the entire biological process from cell division to apoptosis. Moreover, its active ingredient, $1,25(OH)_2D$, can participate in the regulation of neurotransmitter biosynthesis in the central nervous system and regulate cardiovascular autonomic nervous function.[7,8] In addition, Dogdus reported that vitamin D deficiency is independently associated with parasympathetic nerve damage and reduces HRV in healthy adults[9] and patients with type 2 diabetes.[10]

Patients with prediabetes have lower 25(OH)D concentrations than the normal population; however, few studies have explored the correlation between 25(OH)D status and CAN. Therefore, this study determined the relationship between the vitamin D concentration and CAN to provide predictive clinical values pertaining to the occurrence of CVD in patients with prediabetes.

METHODS

Participants

Inclusion criteria

Patients with prediabetes were enrolled between June 2019 and June 2021 from the Department of Endocrinology and Physical Examination Center of the First Hospital of Lanzhou University. On the basis of their standard cardiovascular autonomic reflex test (CARTs) results, 113 patients with prediabetes and definite CAN and 180 with prediabetes but without CAN were enrolled. All the participants met the WHO criteria for the diagnosis of prediabetes. This study was approved by the Ethics Committee of the First Hospital of Lanzhou University, and all investigations were conducted in accordance with the ethical principles of the Declaration of Helsinki.

Exclusion criteria

Patients were excluded if they (1) were diagnosed as having diabetes; (2) had thyroid disease, parathyroid disease, osteoporosis, or other diseases that affected their calcium and phosphorus metabolism; (3) had a history of arrhythmia-related diseases or cardiovascular and cerebrovascular diseases or were taking antiarrhythmic drugs; (4) had been undergoing hypoglycaemic therapy or vitamin D supplementation and drug treatments (e.g., phenobarbital, isoniazid, and corticosteroids) that could affect their serum 25(OH)D concentration; (5) had physical disabilities, insufficient sunlight, or gastrointestinal diseases that affected vitamin D absorption; (6) were older than 80 years, pregnant, or incapable of undergoing Valsalva action or Ewing tests for various reasons; (7) or underwent surgery or had infection, stress, or other complications.

Data collection and laboratory tests

The clinical data of patients who met the inclusion criteria were systematically collected; the collected data comprised sex, age, smoking habit, drinking habit, height, weight, blood pressure (BP), and exercise habit. Fasting plasma glucose (FPG), 2-h postprandial blood glucose (2hPG), total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), HDL, serum calcium, and phosphorus concentrations were measured using an automatic biochemical analyser (Hitachi 7020). Haemoglobin A1C (HbA1C) was measured through high-performance liquid chromatography (Bio-Rad variant turbo II analyser), and the 25(OH)D concentration in serum was measured through chemiluminescence (DiaSorin Stillwater, MN, USA).

CAN and HRV screening

The research procedures were performed by a specially trained clinician, and the participants were asked to refrain from smoking and alcohol and coffee consumption during the 12 h prior to inspection. The CARTs that were conducted comprised (1) Valsalva manoeuvres, (2) examinations of heart rate (HR) responses to deep breathing, (3) examinations of HR responses to standing, (4) examinations of BP responses to standing, and (5) examinations of BP responses to sustained handgrip. For each test, no point was assigned for a normal result, 0.5 point was assigned for a marginally abnormal result, and 1 point was assigned for an abnormal result. A CAN score of 0 indicates the absence of CAN, a score between 0.5 and 1.5 indicates early CAN, a score between 2 and 3 indicates definite CAN, and a score of 3.5 or higher indicates severe CAN.

Twenty-four-hour electrocardiogram (ECG) monitoring was performed using a two-channel (leads CM2 and CM5) amplitude-modulated tape recorder (Diagnostic Monitoring System, Santa Ana, CA, USA). In the present study, the time domain analysis of HRV comprised the assessments of standard deviation of normal-to-normal RR intervals (SDNN), standard deviation of NN interval mean every 5 minutes (SDANN), root mean square of the difference between adjacent RR intervals (RMSSD), and the number of NN intervals > 50ms divided by the percentage of the total number of NN intervals (PNN50%). Spectral measures were computed using the fast Fourier transform method. The frequency domain measurements that were obtained were power in low frequency domain (LF), power in high frequency domain (HF), and LF/HF.

Statistical analysis

The normalised distribution of the measurement data are expressed as ± s. Aa two-sample comparison was performed using an independent samples t test, and a multigroup comparison was performed using ANOVA. M (Q1, Q3) was used to represent the nonnormal distribution of measurement data, the number of cases (percentage) were used to represent dichotomous data, and the χ^2 test or Kruskal–Wallis H test was used for between-group comparisons. Risk factors for CAN were analysed through logistic regression, and the optimal cut-off point for 25(OH)D was determined using a receiver operating characteristic (ROC) curve. All statistical analyses were performed using SPSS 26.0. A p value of <0.05 was regarded as statistically significant.

RESULTS

Baseline characteristics

The baseline characteristics of the participants are listed in Table 1. Age and sex did not significantly differ between the CAN and Non-CAN groups (p>0.05). However, BMI, SBP, TG, LDL, HbA1c, and 2hPG values were significantly greater in the CAN group than in the Non-CAN group (p<0.05). The number of drinkers was significantly greater in the Non-CAN group than in the CAN group (p>0.05). The two groups were significantly different in terms of the 25(OH)D concentration 34.90±10.34 [CAN group] vs. 40.32±13.41 [Non-CAN group], p < 0.05) but not in terms of the duration of prediabetes and the number of people exercising; notably, the duration of prediabetes tended to be long in the CAN group (p>0.05).

CAN scores of participants with different 25(OH)D status

The CARTs revealed that different 25(OH)D groups had different CAN scores; they also indicated that among the 25(OH)D groups, those with 25(OH)D status of ≥75 and <25 nmol/L had the lowest and highest scores, respectively. Among the participants, CAN scores tended to increase with a decrease in the 25(OH)D concentration. Notably, the various groups exhibited significant differences in their heart rate responses to deep breathing (p<0.05) but not for the other test items (Table 2).

Comparison of HRV of participants with different 25(OH)D status

The HRV results obtained through 24-h ECG monitoring are listed in Table 3, which presents the time and frequency thresholds of the participants on the basis of 25(OH)D status. The four 25(OH)D groups exhibited significant differences in SDNN, RMSSD and LF/HF; notably, the group with a 25(OH)D status of <25 nmol/L had significantly lower SDNN and RMSSD relative to the other groups (p<0.05) and the highest LF/HF (p<0.05). Furthermore, when the 25(OH)D concentration increased, SDNN progressively increased, whereas LF/HF gradually decreased. No significant differences in LF, HF, SDANN, PNN50%, and RHR were observed among the four groups (p>0.05).

Correlation analysis of 25(OH)D and HRV

A Pearson correlation analysis of the participants' 25(OH)D and HRV parameters was conducted. As presented in Table 4, 25(OH)D was significantly and positively correlated with SDNN (r=0.652, p<0.05) but significantly and negatively correlated with LF/HF (r=−0.422, p<0.05). The 25(OH)D concentration of the participants was not significantly correlated with LF, HF, SDANN, pNN50%, and RHR (p>0.05).

Regression analysis of factors affecting SDNN and LF/HF in participants with CAN

A multiple linear regression analysis was performed with 25(OH)D, age, BMI, L-LDL, TG, 2hPG, and SBP as independent variables and with SDNN and LF/HF as dependent variables. The results (Table 5) revealed that 25(OH)D was a protective factor for SDNN (β=0.566, p=0.005) but a risk factor for LF/HF (β=−0.199, p=0.012); additionally, LDL was revealed to be a risk factor for SDNN (β=−0.465, p=0.030).

Table 1. Baseline data of patients with prediabetes

Variables	Non-CAN (n=180)	CAN (n=113)	p
n (M/F)	87/93 (48.3/51.7)	60/53 (53.1/46.9)	0.427
Age (years)	57.1±11.3	59.5±9.91	0.369
Height (cm)	167±7.72	167±8.42	0.502
Weight (kg)	67.8±12.4	70.1±13.4	0.074
Ethnic(n)			0.548
Han	160	98	-
Hui	15	9	-
Tibetan	5	6	-
BMI (kg/m^2)	24.24±3.56	25.03±3.27	0.036
SBP (mmHg)	129±18.0	133±16.2	0.048
DBP (mmHg)	79.2±12.3	81.0±11.8	0.129
Smoking, n (%)	70 (38.9)	37 (32.7)	0.288
Alcohol, n (%)	94 (52.2)	69 (61.1)	0.138
25(OH)D (nmol/L)	40.3±13.4	34.9±10.3	0.004
P (mmol/L)	1.26±0.42	1.12±0.21	0.326
Ca (mmol/L)	2.22±0.19	2.20±0.43	0.148
HbA1c (%)	6.08±0.74	6.26±0.77	0.023
FPG (mmol/L)	6.26±0.53	6.31±0.58	0.283
2hPG (mmol/L)	8.64±1.71	8.93±1.25	0.047
TC (mmol/L)	4.23±1.06	4.55±1.17	0.142
HDL (mmol/L)	1.26±0.45	1.24±0.53	0.624
LDL (mmol/L)	2.74±0.83	2.96±0.72	0.046
TG (mmol/L)	1.63±1.21	1.96±1.19	0.038
Creatinine (umol/L)	68.9±20.3	71.6±16.4	0.206
Duration (years)	1.41±0.60	1.80±0.33	0.110
Exercise, n (%)	73 (40.55)	44 (38.93)	0.783

†BMI: body mass index; CAN: cardiac autonomic neuropathy; DBP: diastolic blood pressure; FPG: fasting plasma glucose; F: female; HDL: high-density lipoprotein; LDL: low-density lipoprotein; M: male; Non-CAN: non cardiac autonomic neuropathy; SBP: systolic blood pressure; TC: total cholesterol; TG: triglyceride; 2hPG: 2h postprandial blood glucose.

Table 2. CAN score of different 25(OH)D groups in prediabetes

Variables	25(OH)D≥75 (n=12)	50≤25(OH)D<75 (n=65)	25≤25(OH)D<50 (n=119)	25(OH)D<25 (n=97)	*p*
HR response to deep breathing [beat/min, M (Q1, Q3)]	11 (9, 15)	14 (11, 15)	12 (10, 14)	11 (8, 14)	0.035
HR responses to standing [beat/min, M (Q1, Q]	11 (7, 15)	11 (6, 15)	12 (6, 16)	10 (5, 15)	0.182
BP responses to standing [mmHg, M (Q1, Q]	8 (5, 15)	8 (5, 16)	8 (6, 15)	9 (6, 14)	0.211
BP responses to sustained handgrip [mmHg, M (Q1, Q]	11 (5, 14)	10 (4, 13)	9 (6, 15)	9 (4, 15)	0.251
Valsalva maneuvers [M (Q1, Q]	1.15 (1.12, 1.25)	1.15 (1.10, 1.25)	1.16 (1.12, 1.21)	1.14 (1.08, 1.21)	0.060
CAN score	0	1	1	2	0.112

CARTs: standard Cardiovascular Reflex Test; CAN: cardiac autonomic neuropathy; HR: hart rate; BP: blood pressure.

Table 3. HRV of different 25(OH)D groups in prediabetic CAN

Variables	25(OH)D≥75 (n=4)	50≤25(OH)D<75 (n=18)	25≤25(OH)D<50 (n=47)	25(OH)D<25 (n=45)	*p*
LF (ms^2)	302 (286, 361)	300 (279, 355)	298 (213, 387)	316 (212, 423)	0.860
HF (ms^2)	153 (121, 205)	147 (112, 198)	142 (100, 245)	96.7 (53.6, 153)	0.327
LF/HF	1.4±0.1	1.6±0.2	1.9±0.4†‡	3.1±0.6†‡	0.019
SDNN (ms)	86.3±21.3	83.3±20.9	82.8±19.8†	62.6±20.4†‡§	0.010
SDANN (ms)	73.1±20.8	75.3±20.2	70.8±16.7	61.36±16.8	0.057
RMSSD (ms)	27.0±13.0	27.3±13.5	25.7±12.4†‡	24.5±12.2†‡	0.042
PNN50 (%)	8.0 (7.5, 13.5)	7.7 (6.3, 13.0)	7.4 (3.5, 14.5)	7.3 (3.0, 13.0)	0.423
RHR (beats/min)	73.2±9.2	75.0±8.5	78.3±11.4	79.4±9.3	0.055

SDNN: standard deviation of normal-to-normal RR intervals; SDANN: standard deviation of NN interval mean every 5 minutes; RMSSD: root mean square of the difference between adjacent RR intervals; PNN50%: the number of NN intervals >50ms divided by the percentage of the total number of NN intervals; LF: power in low frequency domain; HF: power in high frequency domain; LF/HF: power ratio between low frequency domain and high frequency domain; RHR: resting heart rate.

†Compared with 25(OH)D ≥75 group, $p<0.05$.

‡Compared with50≤25(OH)D <75 group, $p<0.05$.

§Compared with 25≤25(OH)D <50 group, $p<0.05$.

Table 4. Correlation analysis of 25(OH)D status and HRV in prediabetic CAN

	25(OH)D	
	R	*p*
SDNN (ms)	0.652	0.034
SDANN (ms)	0.193	0.321
RMSSD (ms)	0.382	0.098
PNN50(%)	0.116	0.126
LF (ms^2)	-0.098	0.142
HF (ms^2)	0.341	0.073
LF/HF	-0.422	0.042
RHR (beats/min)	0.127	0.155

SDNN: standard deviation of normal-to-normal RR intervals; SDANN: standard deviation of NN interval mean every 5 minutes; RMSSD: root mean square of the difference between adjacent RR intervals; PNN50%: the number of NN intervals > 50ms divided by the percentage of the total number of NN intervals; LF: power in low frequency domain; HF: power in high frequency domain; LF/HF: power ratio between low frequency domain and high frequency domain; RHR: resting heart rate.

Table 5. Multifactor regression analysis of SDNN, LF/HF in patients with prediabetic CAN

Variables	SDNN		LF/HF	
	B	*p*	*B*	*p*
Age	0.063	0.213	-0.113	0.085
BMI	-0.364	0.128	0.218	0.083
LDL	-0.465	0.030	0.572	0.136
2hPG	0.329	0.416	0.258	0.168
25(OH)D	0.566	0.005	-0.199	0.012
TG	-0.218	0.139	0.172	0.225
SBP	0.372	0.162	0.226	0.051

BMI: body mass index; LDL: low-density lipoprotein; TG: triglyceride; 2hPG=2h postprandial blood glucose; SBP: systolic blood pressure.

Regression model analysis of factors affecting CAN in patients with prediabetes

A regression model was established on the basis of 25(OH)D status (Table 6). Model 1 was a single-factor model. Model 2 controlled for sex, ethnicity, and age on the basis of Model 1. Model 3 controlled for BMI, TG, TC, LDL, BP, and blood glucose on the basis of Model 2. Model 1 revealed that the risk of CAN was significantly increased in the group with 25(OH)D status of <25 nmol/L (OR, 2.320 [1.157–4.450]; $p<0.05$). After adjustments for other possible influencing factors in Model 3, we performed a comparison between the 25(OH)D<25-nmol/L and 50≤25(OH)D＜75-nmol/L groups. The results indicated that the risk of CAN was increased by 2.380 in the 25(OH)D<25-nmol/L group (adjusted OR, 2.380 [1.208-4.691]; $p<0.05$) and by 1.875 in the 25≤25(OH)D<50-nmol/L group (adjusted OR, 1.875 [1.064-3.751] p<0.05). Notably, the risk of CAN was higher in the 25(OH)D ≥75-nmol/L group than in the 20≤25(OH)D＜75-nmol/L group ($p>0.05$); however, this difference was nonsignificant.

ROC curve analysis of vitamin D cut-off point

ROC curve analysis revealed that the optimal 25(OH)D cut-off point for predicting CAN in individuals with prediabetes was 25.65 nmol/L; the area under the curve was 0.530, and the sensitivity and specificity were 66.4% and 86.7%, respectively (Figure 1).

DISCUSSION

The increasing prevalence of diabetic CVD is increasingly attracting attention from scholars. Studies have focused on the relationship between CAN and diabetes; however, Fraser et al reported early peripheral neuropathy and early damage to cardiac autonomic nerves in patients newly diagnosed as having diabetes, and they suggested that this is related to nerve fibre demyelination caused by the interference of Schwann cells.[11] Moreover, Sanyal et al indicated that streptozotocin-induced early diabetic rats with poor blood glucose control exhibited an imbalance in the interaction between their cholinergic and adrenergic neurons and changes in their cardiac autonomic nerve ultrastructure (e.g., axoplasmic disintegration, axonal cell degeneration, and synaptic or terminal degeneration of

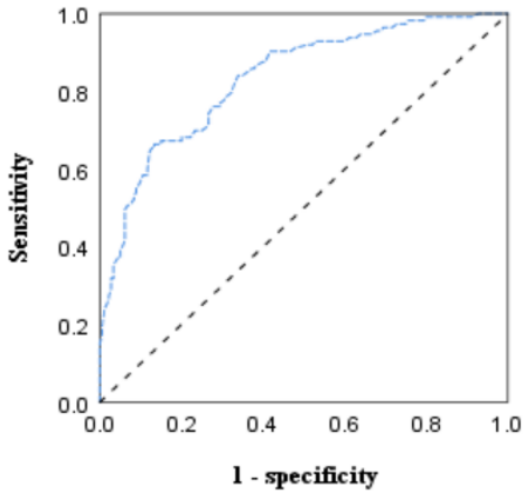

Figure 1. ROC curve of serum 25(OH)D in predicting prediabetic CAN. ROC: receiver operating characteristic; CAN: cardiac autonomic neuropathy.

Table 6. Logistic regression model of correlation between vitamin D and CAN in prediabetes

Groups (ng/ml)	B	SE	Wald	*p*	OR	95%CI
Model 1						
50≤25(OH)D<75			5.798	0.122	1 (reference)	
25(OH)D<25	0.815	0.344	5.618	0.018	2.260	1.152-4.434
25≤25(OH)D<50	0.533	0.335	2.539	0.052	1.704	0.885-3.284
25-OH-D≥75	0.267	0.672	0.157	0.237	1.306	0.350-4.875
Model 2						
50≤25(OH)D<75			5.922	0.109	1 (reference)	
25(OH)D<25	0.842	0.355	5.627	0.024	2.320	1.157-4.450
25≤25(OH)D<50	0.526	0.353	2.224	0.069	1.692	0.848-3.379
25(OH)D≥75	0.254	0.714	0.127	0.126	1.289	0.318-4.225
Model 3						
50≤25(OH)D<75			6.292	0.046	1 (reference)	
25(OH)D<25	0.867	0.346	6.281	0.019	2.380	1.208-4.691
25≤25(OH)D<50	0.619	0.359	2.979	0.043	1.875	1.064-3.751
25(OH)D≥75	0.297	0.674	0.194	0.120	1.345	0.309-5.346

Model 1: Single factor model.
Model 2: Gender, ethnic and age were controlled based on Model 1.
Model 3: BMI, TG, TC, LDL, blood pressure and blood glucose were corrected on the basis of Model 2.

cardiac autonomic nerves), which subsequently led to CAN.[12,13] A study reported that CAN progresses significantly in the 2 years after diagnosis; therefore, an early diagnosis is crucial for preventing this disease.[14] The aforementioned evidence suggests that autonomic neuropathy exists in the early stage of diabetes; thus, the monitoring of CAN in patients with prediabetes warrants clinical attention.

Prediabetes is correlated with vitamin D status, and the corresponding mechanism is attributed to the presence of vitamin D receptors, vitamin D binding proteins, and 1-α-hydroxylase in pancreatic β cells.[6,15] The deficiency or insufficiency of 25(OH)D can change the normal metabolic function of a body and induce the occurrence of IFG and IGT; populations with a low 25(OH)D concentration have an increased risk of impaired glucose metabolism or diabetes. Studies have identified a link between vitamin D status and diabetic neuropathy. Sternberg suggested that low vitamin D status can stimulate the production of adrenal medulla catecholamine and vitamin D may cross the blood–brain barrier through adrenergic neurons (which are rich in vitamin D nuclear receptors) located in the spinal cord and brain stem to regulate the activity of the autonomic nervous system.[16] Larsson et al reported that an interactive signalling pathway exists between vitamin D receptors and peroxisome proliferator-activated receptors (PPARs), and they suggested that vitamin D improves neuronal peroxisome function and the metabolism of neuronal lipid intermediates through PPARγ activation, thus exerting a protective effect on neurons.[17] Therefore, 25(OH)D status may affect autonomic neuropathy through various pathways in individuals with prediabetes.

At the time of writing, the diagnostic criteria for vitamin D deficiency are inconsistent. In the Endocrine Society's Clinical Practice Guidelines, Michael et al suggested a 25(OH)D concentration of <50 nmol/L as the diagnostic criterion for the general population.[2] However, in the vitamin D supplementation Guidelines, Pludowski et al highlighted the pleiotropic effects of vitamin D and recommended a target 25(OH)D concentration of 75 nmol/L.[18] In our study, patients with prediabetes were recruited, and the prevalence of vitamin D deficiency was observed to be 73.7% (diagnostic criterion, 25(OH)D <50 nmol/L), which is considerably higher than the 30%–50% range for the general population. This finding suggests that vitamin D deficiency is a risk factor for prediabetes. In addition, we performed noninvasive tests to evaluate the CAN of patients with prediabetes and discovered that low 25(OH)D status was associated with the occurrence of CAN, although this relationship was nonlinear. Alamdari et al reported that serum vitamin D status is highly correlated with diabetic peripheral neuropathy and CAN; specifically, a 2.5-nmol/L increase in the serum 25(OH)D concentration caused the presence and severity of nerve conduction velocity impairment to decrease by 2.2% and 3.4%, respectively.[19] Mann et al examined the vitamin D concentrations of multiple groups and identified a severe autonomic nerve imbalance in the group with the lowest vitamin D concentration; they also reported that vagus nerve inhibition in healthy people can be reduced through vitamin D3 supplementation.[20] An *in vivo* study revealed that compared with control group rats, rats with vitamin D deficiency exhibited strengthened ventricular and vascular muscular systole function and greater sensitivity to the major sympathetic neurotransmitter norepinephrine.[21] Our finding is consistent with the aforementioned findings; that is, low vitamin D status correlates closely with CAN, may impair sympathetic and vagus nerve balance, and increases the risk of CVD in patients with both prediabetes and vitamin D deficiency.

We evaluated the correlation between HRV and serum 25(OH)D in patients with prediabetes, and we discovered that 25(OH)D is positively correlated with SDNN but negatively correlated with LF/HF. HRV is an effective indicator that reflects the state of the cardiac autonomic nervous system. SDNN is affected by adrenergic and cholinergic activities and reflects the overall tension of cardiac autonomic nerves, RMSSD and HF reflect parasympathetic nerve activity, and LF/HF reflects autonomic nerve balance. The present study revealed that both sympathetic and parasympathetic nerves are impaired in individuals with prediabetes and CAN; however, the dominance of

parasympathetic nerves relative to sympathetic nerves may be related to the attenuation of vagal ganglion transmission, changes in muscarinic receptor composition and density, and a decrease in acetylcholinesterase activity. The findings of Agashe et al are consistent with our results; that is, CAN mainly damages parasympathetic nerves in the early stage, particularly the longest vagus nerve in the body that is responsible for 75% of parasympathetic activity.[22] The loss of parasympathetic tone is accompanied by compensatory increases in sympathetic tone, which increase norepinephrine status, increase insulin resistance, and reduce cardiac efficiency, leading to resting tachycardia.[23] When CAN progresses, sympathetic or parasympathetic nerve damage gradually worsens and is followed by sympathetic denervation and postural hypotension.[22] Tak et al analysed the serum 25(OH)D and HRV indices of 173 healthy individuals and reported that the status of 25(OH)D was positively correlated with SDNN and LF but not significantly correlated with HF; this finding suggests that the occurrence of CAN in patients with vitamin D deficiency is mainly mediated by a decrease in sympathetic nerve activity.[24] The aforementioned studies revealed that vitamin D status is related to several indicators of HRV and that vitamin D deficiency causes HRV changes and cardiac autonomic nervous system imbalance; these findings are consistent with our research results. However, we discovered that vitamin D affects both sympathetic and parasympathetic nerves but main damaged parasympathetic nerves. This result is inconsistent with that reported by Tak, which may be due to differences in population characteristics (healthy population vs. prediabetic population).

In addition to hyperglycaemia, other factors can affect diabetic autonomic neuropathy; they include age, gender, ethnicity, smoking habit, obesity, hypertension, hyperlipidaemia, and hormone disorders. In the present study, a logistic regression model was established and stratified by 25(OH)D status, and confounding factors were gradually controlled through adjusted models in which the 50≤25(OH)D<75-nmol/L group was used as the reference. The results indicate an increased risk of CAN in the 25(OH)D<50-nmol/L and 25≤25(OH)D<50-nmol/L groups, especially the 25(OH)D<25-nmol/L group; this finding suggests that serum 25(OH)D is inversely associated with CAN in individuals with prediabetes. The mechanism by which low 25(OH)D leads to CAN is still unclear, and the current opinion is that the mechanism may be related to the body's autoimmune regulation disorder;[25] vitamin D deficiency hinders the regulation of dendritic cells and Th1 lymphocytes cells, thereby impairing the ability of patients with prediabetes to tolerance complications and increasing their risk of complications. Studies have also suggested that vitamin D deficiency can damage sympathetic and parasympathetic nerves through oxidative stress, and the exogenous supplementation of vitamin D may provide considerable protective effects against vascular and neurological complications mediated by oxidative stress in diabetic model rats.[26] In addition, basic and clinical studies have suggested that vitamin D reduces catecholamine synthesis by affecting the expression of tyrosine hydroxylase gene[27] and negatively affects sympathetic regulation. Vitamin D deficiency causes sympathetic tension to increase, leading to baroreceptor and cardiac vagal tension inhibition and, consequently, autonomic nervous function imbalance.

Notably, we discovered an increasing risk in the prevalence of CAN in the 25(OH)D ≥75-nmol/L group (OR=1.345). This finding may be related to the following mechanisms. Elevated plasma and intracellular 25(OH)D concentration stimulate intracellular 24-hydroxylase (CYP24A1), which catalyses the conversion of 1,25(OH)2D and 25(OH)D into bioinactive water-soluble metabolites, which are then excreted into bile, leading to a decrease in intracellular 1,25(OH)2D concentration. Therefore, this process induces a pathogenesis that resembles that of vitamin D deficiency.[18,28] In our study, the relationship between vitamin D status and CAN is inverted and U-shaped, and an excessively high or low vitamin D status has adverse effects; this finding is consistent with the results of Hansen's study.[29]

We detected a significant increase in LDL status in the CAN group, and a linear regression revealed a negative correlation between LDL and SDNN, indicating that LDL is related to autonomic nerve imbalance, HRV, and CAN occurrence. Large-scale clinical studies have verified that dyslipidaemia and obesity are common risk factors for CAN and hyperglycemia.[30,31] In addition, Chang et al suggested that all individual components of metabolic syndrome are presented as a reduced parasympathetic modulation of the heart (through SDNN and E/I).[32] The aforementioned results are consistent with those of our study. On the other hand, researchers have reported that the prevalence of vitamin D deficiency increases with age. However, age and gender differences pertaining to CAN were not identified in our study, which may be related to the demographics of our study population; that is, our study population comprised middle-aged and older patients, and their overall age span was small.

Studies have revealed that vitamin D supplementation can improve FBG and glycosylated haemoglobin in patients with diabetes and that a close relationship exists between vitamin D and HRV parameters. However, few studies have explored the relationship between vitamin D and prediabetic CAN. We investigated the relationship between 25(OH)D concentration and CAN in individuals with prediabetes but did not explore the causality of this relationship. Subsequent studies should focus on the effects of vitamin D supplementation in patients with prediabetes and CAN.

Conclusion

The present study revealed a significant correlation between serum 25(OH)D and prediabetic CAN. SDNN and LF/HF are positively and negatively correlated with 25(OH)D status, respectively, and increased LF/HF is a risk factor for CAN in individuals with prediabetes. The ROC curve analysis revealed that the optimal 25(OH)D cut-off point for predicting CAN is 25.65 nmol/L. This finding suggests that low vitamin D status is related to the occurrence of CAN in individuals with prediabetes; therefore, appropriate supplementation may have a protective effect against cardiac autonomic nerves.

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AUTHOR DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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