Am J Transl Res 2024;16(8):3759-3768
www.ajtr.org /ISSN:1943-8141/AJTR0157482

Original Article

Correlation between serum 25-hydroxyvitamin D level and peripheral arterial disease in patients with type 2 diabetes mellitus: a single-center retrospective study

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Received April 28, 2024; Accepted June 23, 2024; Epub August 15, 2024; Published August 30, 2024

Abstract: Objective: To investigate the association between serum 25-hydroxyvitamin D (25(OH)D) level and peripheral arterial disease (PAD) in patients with type 2 diabetes mellitus (T2DM). Methods: This retrospective study analyzed data from 752 T2DM patients treated at Shaoyang Central Hospital between September 2020 and September 2023. Patients were divided into two groups: those with T2DM alone and those with T2DM and PAD. We compared demographic data, biochemical indices, and ankle-brachial index (ABI) values. Pearson correlation and multivariate logistic regression with a forward likelihood ratio method assessed the relationship and risk factors. The predictive value of serum 25(OH)D levels for PAD was evaluated using receiver operating characteristic (ROC) analysis. Results: The T2DM+PAD group was older and had a longer duration of diabetes compared to the T2DM group. This group also had lower BMI, diastolic blood pressure, and ABI values, but higher levels of low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) (all $P<0.05$). Serum 25(OH)D levels were significantly lower in the T2DM+PAD group ($P<0.05$). ABI negatively correlated with age, diabetes duration, LDL-C, and TC, and positively with BMI and 25(OH)D levels (all $P<0.05$). Older age, lower BMI, higher LDL-C, and lower 25(OH)D levels were independent risk factors for PAD (ORs: 1.060, 0.781, 1.083, and 0.959, respectively; all $P<0.05$). The risk of PAD was significantly higher in the 25(OH)D deficiency group ($P<0.05$). The AUC for serum 25(OH)D in predicting PAD occurrence was 0.629. Conclusion: Lower serum 25(OH)D levels are associated with higher risk of PAD in patients with T2DM. Early identification and management of 25(OH)D deficiency may be crucial for preventing PAD in this population.

Keywords: Type 2 diabetes, peripheral arterial disease, 25-hydroxyvitamin D, ankle brachial index, atherosclerosis

Introduction

Type 2 diabetes mellitus (T2DM) is the most common form of diabetes and poses a significant global health challenge. The prevalence of T2DM-related complications, such as peripheral arterial disease (PAD), is increasing alongside the disease's incidence [1]. PAD, primarily caused by atherosclerosis (AS), often leads to severe complications including stenosis or occlusion of the leg arteries, resulting in ischemia and diverse clinical symptoms in the lower extremities [2, 3]. Early stages of PAD may present with atypical symptoms like reduced skin temperature, edema, and numbness in the limbs. As the disease progresses, more severe symptoms such as intermittent claudication, resting pain, ischemic ulcers, and gangrene can develop, causing morbidity and mortality [4, 5]. Thus, early detection of PAD risk factors in patients with T2DM is crucial for timely and accurate intervention.

25-hydroxyvitamin D (25(OH)D) plays a critical role in several physiologic processes, including endocrine regulation and inflammatory responses. This fat-soluble vitamin is vital for glucose and lipid metabolism, calcium and phosphorus homeostasis, and bone health [6, 7]. Deficiencies in 25(OH)D have been associated with increased risks of various diseases, including osteoporosis, immune disorders, PAD, and other cardiovascular diseases [8, 9].

Recent studies, including one by Li et al., which examined 1028 T2DM patients, have highlight-

https://doi.org/10.62347/YOBS2983

ed the strong correlation between low serum 25(OH)D level and increased PAD incidence in individuals under 65 [10]. Despite growing interest in the impact of 25(OH)D levels on PAD risk, comprehensive research in this area remains limited, particularly regarding the effects of varying 25(OH)D levels on PAD development.

This study aims to fill this research gap by conducting a thorough analysis of the association between serum 25(OH)D levels and the occurrence of PAD in a retrospective cohort of 752 T2DM patients, thereby contributing to early prevention strategies for PAD.

Materials and methods

Research subjects

This retrospective study analyzed data from 752 patients with T2DM treated at Shaoyang Central Hospital from September 2020 to September 2023. These patients were categorized into 673 in the T2DM group and 79 in the T2DM+PAD group. Ethical approval was granted by the Ethics Committee of Shaoyang Central Hospital.

Inclusion criteria: Diagnosis of diabetes according to World Health Organization criteria [11]; Patients ≥18 years old; Availability of complete clinical data including chief complaints, family history, physical examination, and biochemical indices.

Exclusion criteria: Presence of conditions affecting vitamin D metabolism, such as chronic renal insufficiency, malabsorption syndrome, or abnormal bone metabolism; Regular use of vitamin D or calcium supplements; Specific or complicated forms of diabetes including diabetic ketoacidosis, hyperosmotic hyperglycemia syndrome, and hypoglycemic coma, Chronic gastrointestinal diseases, malignancies, serious infections, acute cardiovascular or cerebrovascular events, thyroid or parathyroid disorders, autoimmune or mental health disorders; Pregnant or lactating women.

Data collection

Demographic and clinical data collected included gender, age, duration of diabetes, family history of diabetes, smoking status (defined as continuous or cumulative smoking for 6 months or more), and alcohol consumption (exceeding 60 g/day for males and 40 g/day for females). Physical examinations were conducted to measure height, weight, systolic and diastolic blood pressures (SBP and DBP) after a 20-minute rest using a standard mercury sphygmomanometer, with the average of two measurements recorded. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

Biochemical indices collection

Peripheral venous blood samples (5 mL) were collected after an overnight fast (≥12 hours), centrifuged at 3000 rpm for 15 minutes, and stored at -20°C. Biochemical indices analyzed included hemoglobin A1c (HbA1c), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), and 25(OH)D levels.

Patients were classified into three groups based on their serum 25(OH)D levels: 25(OH)D deficiency: <20 ng/Ml; 25(OH)D insufficiency: 20 ng/mL≤25(OH)D<30 ng/mL; 25(OH)D sufficiency: ≥30 ng/ml.

Ankle brachial index (ABI) measurement

ABI measurements were conducted by a trained technician using Doppler ultrasound and a portable optical volume detector. Patients rested in the supine position for 5 minutes before the cuff was positioned on the medial ankle and the upper arm's brachial artery. The ultrasound probe was adjusted along the artery bend until optimal signals were obtained, and SBP was measured at both the ankles and upper arms. The ABI was calculated as the ratio of ankle SBP to brachial arterial SBP. ABI values below 0.9 on either side indicated significant PAD, classifying patients into the T2DM+PAD group [12].

Statistical analysis

Data were analyzed using SPSS software version 24.0. The distribution of data was assessed with the Kolmogorov-Smirnov test. Normally distributed data were presented as mean ± SD, and differences between groups were evaluated using the independent samples t-test. Categorical data were analyzed using the χ^2 test or Fisher's exact test as appropriate. Pearson's correlation coefficient was used to

Table 1. Comparison of basic data ($\bar{x}\pm s$)/[n (%)]

Group	T2DM group (n = 673)	T2DM+PAD group (n = 79)	χ^2/t value	*P* value
Sex			0.001	0.973
Male	382 (56.76%)	45 (56.96%)		
Female	291 (43.24%)	34 (43.04%)		
Age (years)	60.23±11.69	66.70±11.50	4.658	<0.001
Course of diabetes mellitus (years)	8.31±6.93	11.78±7.79	4.149	<0.001
Family history of diabetes mellitus			2.042	0.153
With	17 (2.53%)	0 (0.00%)		
Without	656 (97.47%)	79 (100.00%)		
History of smoking			0.170	0.680
With	99 (14.71%)	13 (16.46%)		
Without	574 (85.29%)	66 (83.54%)		
History of alcoholism			-	0.792*
With	38 (5.65%)	3 (3.80%)		
Without	635 (94.35%)	76 (96.20%)		
BMI (kg/m^2)	24.12±3.66	22.68±3.20	3.334	0.001
SBP (mmHg)	134.30±19.26	134.82±19.45	0.228	0.820
DBP (mmHg)	81.07±11.62	77.87±11.72	2.314	0.021
ABI-R	1.11±0.10	0.83±0.19	19.950	<0.001
ABI-L	1.10±0.10	0.85±0.20	18.645	<0.001

Note: *Fisher's exact test. t: data from t-test; χ^2: data from chi-square test. T2DM: type 2 diabetes mellitus; PAD: peripheral arterial disease; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; ABI-R: ankle brachial index-right; ABI-L: ankle brachial index-left.

explore relationships between variables. A forward stepwise logistic regression model identified independent predictors of PAD, and ROC analysis assessed the predictive value of serum 25(OH)D levels. A *P*-value of <0.05 was considered significant.

Results

Comparison of basic data

The mean age was significantly higher in the T2DM+PAD group (66.70±11.50 years) compared to the T2DM group (60.23±11.69 years). The duration of diabetes was also longer in the T2DM+PAD group (11.78±7.79 years) versus the T2DM group (8.31±6.93 years) (all P<0.05). The T2DM+PAD group showed significantly lower BMI (22.68±3.20 kg/m^2), DBP (77.87±11.72 mmHg), and ABI values (ABI-R: 0.83±0.19, ABI-L: 0.85±0.20) compared to the T2DM group (all P<0.05). There were no significant differences in sex, family history of diabetes, smoking history, alcohol consumption, or SBP between the two groups (all P>0.05). See **Table 1**.

Comparison of biochemical indices

Biochemical markers such as HbA1c, TG, LDL-C, HDL-C, TC and 25(OH)D, were analyzed for both groups. Serum levels of LDL-C and TC were significantly higher in the T2DM+PAD group [(3.11±1.70) mmol/L and (4.97±1.63) mmol/L, respectively] compared to the T2DM group [(2.64±1.02) mmol/L and (4.64±1.30) mmol/L, respectively]. Serum 25(OH)D levels were lower in the T2DM+PAD group [(20.29±8.69) ng/mL] than in the T2DM group [(24.88±9.72) ng/mL] (all P<0.05). No significant differences were observed in serum levels of HbA1c, TG, or HDL-C between the groups (all P>0.05). See **Table 2**.

Correlation between ABI and other variables

ABI-R was negatively correlated with age, duration of diabetes, LDL-C, and TC (r = -0.097, r = -0.092, r = -0.117 and r = -0.075, respectively; all P<0.05), and positively correlated with BMI and 25(OH)D (r = 0.131 and r = 0.156, respectively; all P<0.05). Similarly, ABI-L demonstrated negative correlations with age and duration

Table 2. Comparison of biochemical indices ($\bar{x}\pm s$)

Group	T2DM group (n = 673)	T2DM+PAD group (n = 79)	*t* value	*P* value
HbA1c (%)	9.49±2.43	9.44±2.21	0.174	0.862
TG (mmol/L)	2.43±3.45	2.08±1.94	0.891	0.373
LDL-C (mmol/L)	2.64±1.02	3.11±1.70	3.551	<0.001
HDL-C (mmol/L)	1.22±0.74	1.26±1.01	0.374	0.709
TC (mmol/L)	4.64±1.30	4.97±1.63	2.029	0.043
25(OH)D (ng/mL)	24.88±9.72	20.29±8.69	4.013	<0.001

Note: t: data from t-test. T2DM: type 2 diabetes mellitus; PAD: peripheral arterial disease; HbA1c: hemoglobin A1C; TG: triglyceride; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; TC: total cholesterol; 25(OH)D: 25-hydroxyvitamin D.

of diabetes (r = -0.150 and r = -0.114, respectively; all P<0.05), and positive correlations with BMI and 25(OH)D (r = 0.076 and r = 0.127, respectively; all P<0.05). See **Figures 1** and **2**.

Multivariate analysis of PAD risk factors

Multivariate logistic regression analysis identified age, BMI, LDL-C, and 25(OH)D as independent risk factors for PAD in T2DM patients. The odds ratios (OR) and 95% confidence intervals (CI) were as follows: age OR = 0.959 (95% CI: 0.935-0.983), BMI OR = 1.083 (95% CI: 1.005-1.168), LDL-C OR = 0.781 (95% CI: 0.616-0.991), and 25(OH)D OR = 1.060 (95% CI: 1.029-1.092) (all P<0.05). See **Table 3**.

Comparison of PAD incidence across different 25(OH)D levels

The incidence of PAD varied significantly with different levels of 25(OH)D deficiency. The probability of PAD in the 25(OH)D deficiency group (15.27%) was substantially higher compared to the sufficiency group (5.49%) (P<0.05). See **Table 4**.

Predictive value of 25(OH)D in PAD in T2DM patients

The ROC analysis revealed that the area under the curve (AUC) for serum 25(OH)D that predicted PAD in patients with T2DM was 0.629. The optimal cutoff value determined was 21.65 ng/mL, which provided a sensitivity of 60.3% and a specificity of 63.3% for diagnosing PAD, as illustrated in **Figure 3**.

Discussion

It is widely recognized that the development of PAD is influenced by factors such as arterial hypertension, obesity, oxidative stress, inflammatory response, insulin resistance, and dyslipidemia [13]. PAD-associated lesions typically involve large and medium arteries, but also extend to small and medium-sized arteries below the knee, potentially increasing the risk of cerebrovascular and cardiovascular diseases [14]. Early screening for PAD is therefore crucial.

The ABI is frequently employed due to its quick, simple, inexpensive, and non-invasive nature, serving as a reliable measure of PAD severity [15]. In this study, ABI was positively correlated with BMI and negatively correlated with age, duration of diabetes, LDL-C, and TC. Identified risk factors for PAD in T2DM patients include older age, lower BMI, and higher LDL-C. With aging and prolonged diabetes duration, T2DM patients often develop arterial calcifications in the lower extremities [16]. Prolonged hyperglycemia accelerates AS progression, resulting in PAD by increasing the formation of advanced glycation end products, which activate the hexosamine biosynthesis pathway and damage the vascular endothelium [17]. Dyslipidemia in T2DM can lead to increased cholesterol esterification and reduced free cholesterol levels, promoting excessive accumulation of cholesterol esters, facilitating LDL-C transport in endothelial cells, and accelerating foam cell formation, thereby impairing vascular endothelial function and increasing PAD risk [18]. Additionally, low BMI is associated with an increased risk of vascular diseases, linking emaciation to insulin resistance and metabolic disturbances in T2DM patients [19]. Consequently, it is essential to prioritize screening and appropriate interventions for elderly T2DM patients, those with a prolonged course of the disease, those with dyslipidemia, or

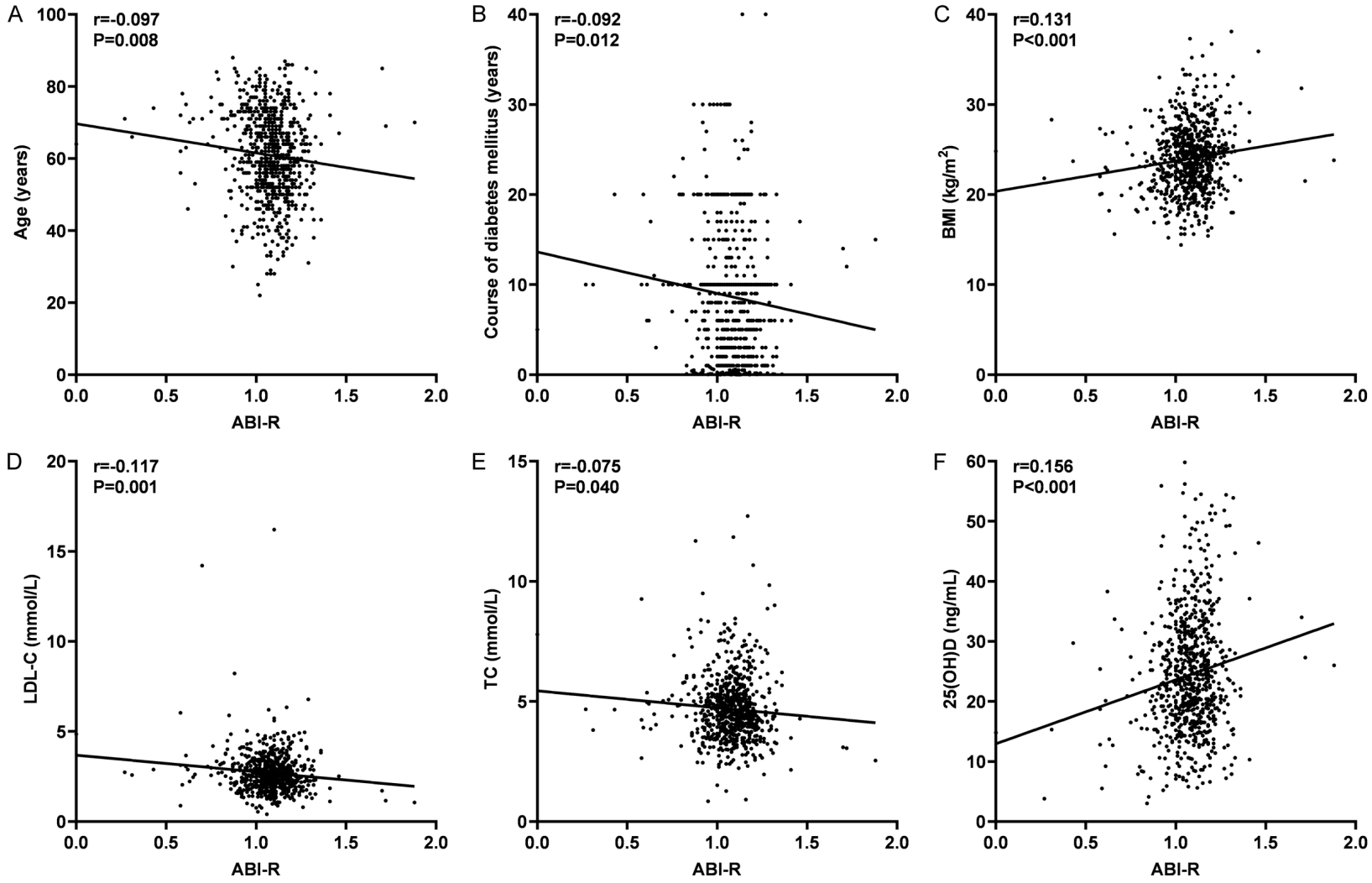

Figure 1. Correlation between ABI-R and other variables. A. Age; B. Course of diabetes mellitus; C. BMI; D. LDL-C level; E. TC level; F. 25(OH)D level. ABI-R: ankle brachial index-right; BMI: body mass index; LDL-C: low density lipoprotein cholesterol; TC: total cholesterol; 25(OH)D: 25-hydroxyvitamin D.

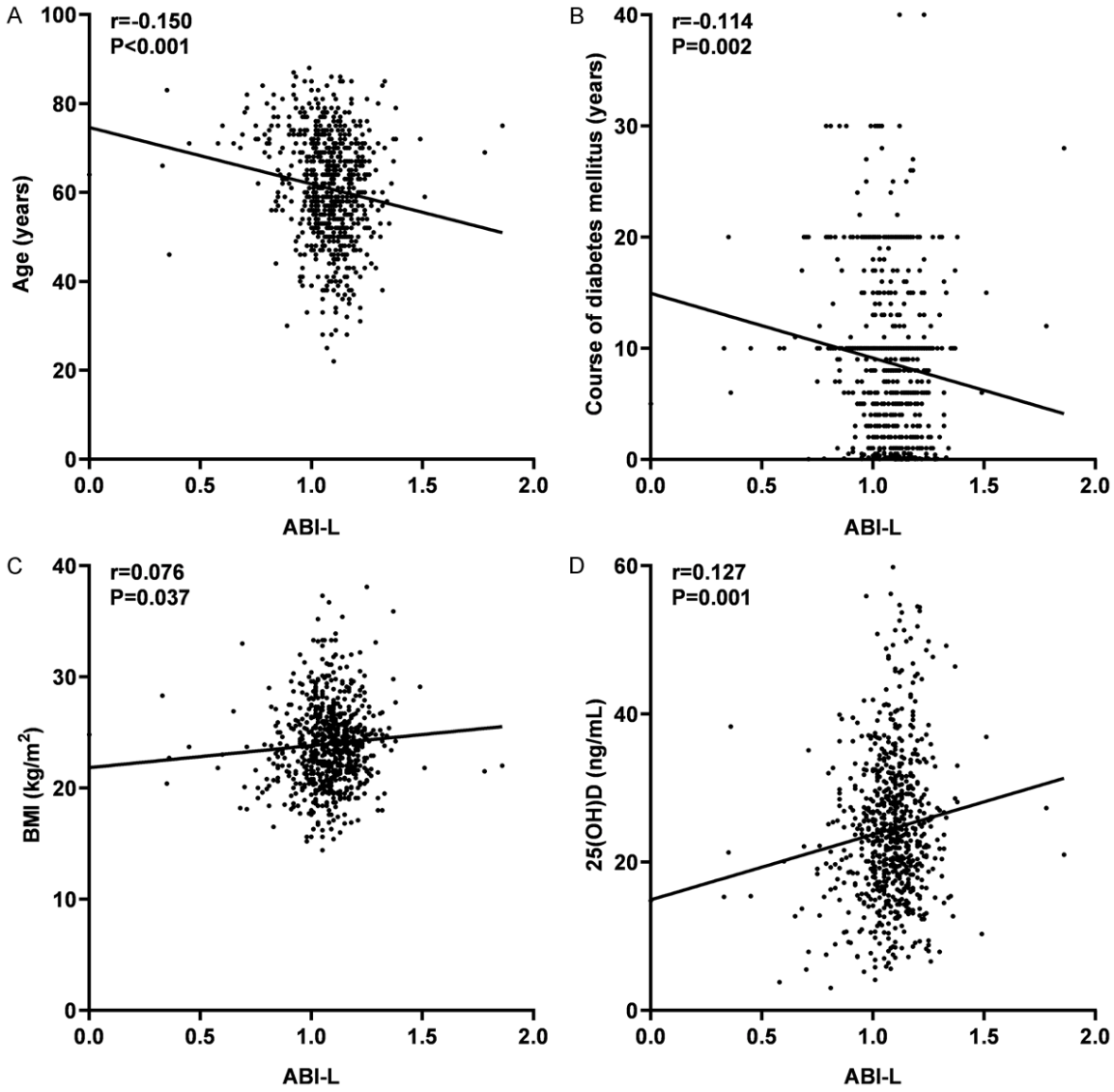

Figure 2. Correlation between ABI-L and other variables. A. Age; B. Course of diabetes mellitus; C. BMI; D. 25(OH)D level. ABI-L: ankle brachial index-left; BMI: body mass index; 25(OH)D: 25-hydroxyvitamin D.

Table 3. Multivariate analysis on the occurrence of PAD in T2DM patients

Variable	B	S.E.	Walds χ^2	*P*	Exp (B)	Exp (B) 95% C.I.	
						Lower limit	Upper limit
Age	-0.042	0.013	10.701	0.001	0.959	0.935	0.983
Course of diabetes mellitus	-0.026	0.018	2.163	0.141	0.974	0.941	1.009
BMI	0.080	0.038	4.318	0.038	1.083	1.005	1.168
DBP	0.017	0.012	2.058	0.151	1.017	0.994	1.041
LDL-C	-0.247	0.121	4.154	0.042	0.781	0.616	0.991
TC	-0.069	0.114	0.360	0.549	0.934	0.746	1.168
25(OH)D	0.058	0.015	14.669	<0.001	1.060	1.029	1.092

Note: T2DM: type 2 diabetes mellitus; PAD: peripheral arterial disease; BMI: body mass index; DBP: diastolic blood pressure; LDL-C: low density lipoprotein cholesterol; TC: total cholesterol; 25(OH)D: 25-hydroxyvitamin D.

Table 4. Comparison of PAD occurred in different degrees of 25(OH)D deficiency (n, %)

Group	25(OH)D deficiency group (n = 262)	25(OH)D insufficiency group (n = 308)	25(OH)D sufficiency group (n = 182)	χ^2 value	*P* value
T2DM	222 (84.73%)	279 (90.58%)	172 (94.51%)	11.569	0.003
T2DM+PAD	40 (15.27%)	29 (9.42%)	10 (5.49%)		

Note: χ^2: data from chi-square test. T2DM: type 2 diabetes mellitus; PAD: peripheral arterial disease; 25(OH)D: 25-hydroxyvitamin D.

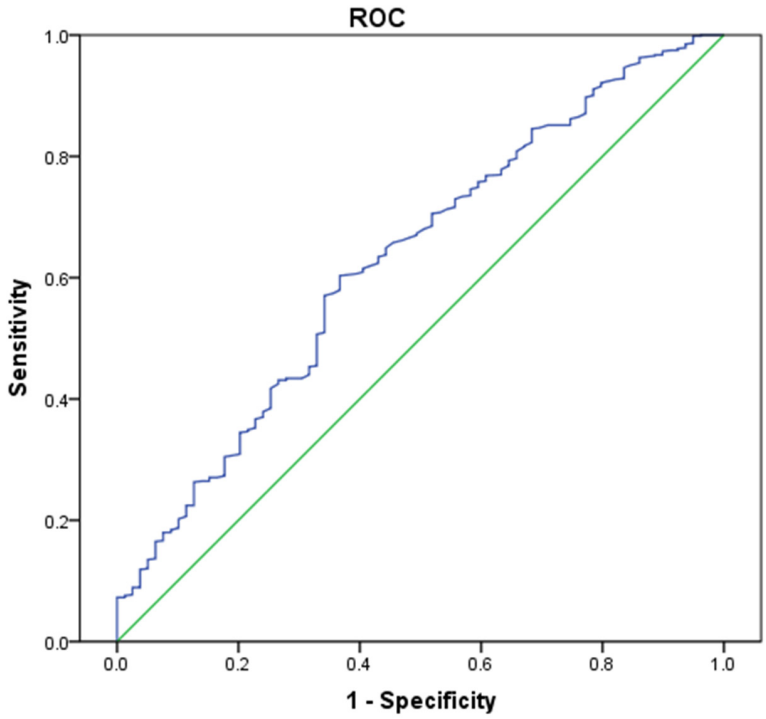

Figure 3. Value of 25(OH)D in predicting the PAD occurred in T2DM patients. ROC: receiver operating characteristic; PAD: peripheral arterial disease.

those who are underweight. 25(OH)D not only ensures bone health but also plays crucial roles in immunity regulation, glycemic control, and cellular processes including proliferation, differentiation, and apoptosis [20, 21]. Dziedzic et al. reported that women with severe coronary AS exhibited low serum levels of 25(OH)D, indicating a correlation with the severity of coronary AS [22]. In a study involving 603 T2DM patients, Ma et al. found that those with AS had a higher prevalence of 25(OH)D deficiency, and there was a negative correlation between brachial-ankle pulse wave velocity and 25(OH)D levels [23]. Stančáková et al. observed that a decrease of 10 ng/mL in 25(OH)D levels was associated with a doubling of all-cause mortality risk, highlighting its impact on cardiovascular events and mortality in T2DM patients [24]. Similarly, Rapson et al. identified an increased risk of PAD associated with 25(OH)D deficiency in both Black and white adults [25].

In our research, both ABI-R and ABI-L demonstrated positive correlations with 25(OH)D levels; low levels of 25(OH)D were significant risk factors for PAD in T2DM patients. Receiver operating characteristic (ROC) analysis indicated that the AUC for serum 25(OH)D in predicting PAD was 0.629. These findings suggest that decreased serum 25(OH)D levels are linked to an increased risk of PAD, suggesting serum 25(OH)D as a marker for PAD risk in T2DM patients. Moreover, when categorizing patients based on their 25(OH)D levels, the PAD occurrence rate in the 25(OH)D deficiency group was significantly higher compared to the sufficiency group, further emphasizing that the risk of PAD escalates with declining 25(OH)D levels. The author proposes several mechanisms by which 25(OH)D may influence the development of PAD in patients with T2DM.

Vascular Calcification and Endothelial Function: Prolonged hyperglycemia in T2DM patients can disrupt calcium and phosphorus balance, leading to calcium deposition in blood vessels and endothelial damage. 25(OH)D may regulate the expression of vascular endothelial growth factor, matrix metalloproteinase-9, elastin, and myosin, which are critical to arterial wall integrity. It also inhibits coagulation factors and smooth muscle cell proliferation, thus protecting against arterial calcification and maintaining vascular function [26, 27].

25(OH)D modulates immune activity by regulating lymphocytes and macrophages, reducing the secretion of pro-inflammatory cytokines such as interleukin-6 and C-reactive protein, and enhancing the secretion of anti-inflammatory interleukin-10. These actions help mitigate chronic arterial wall inflammation and reduce the formation of foam cells, potentially decreasing the incidence of PAD [28]. Renin-Angiotensin-Aldosterone System (RAAS) Overactivity: A deficiency in 25(OH)D can lead to over-activation of the RAAS, increasing angiotensin expression, which enhances vasoconstriction, promotes matrix remodeling, and escalates oxidative stress. These changes contribute to AS and accelerate PAD progression [29]. High levels of 25(OH)D may inhibit the formation of AS and prevent PAD by reducing the expression of parathyroid hormone-related peptide and subsequent calcium release, thereby controlling the pathologic calcification processes [30].

It is important to note that some studies suggest serum 25(OH)D may not significantly affect PAD development in T2DM patients [31]. Such discrepancies could be due to variations in patient population, geographic region, research methodology, or other factors. This indicates the need for further research in diverse settings to clarify these relationships.

Recent reviews have consolidated findings from multiple studies regarding the prevalence of PAD and its association with vitamin D levels. A meta-analysis of 15 studies highlighted a significant correlation between vitamin D deficiency and the incidence of PAD, underscoring reduced vitamin D levels as an independent risk factor for PAD [32]. Building on this foundation, the author's study introduces innovative methodologies to further explore the relationship between serum 25(OH)D levels and PAD occurrence.

The study employed a retrospective analysis of medical records to assess the factors influencing PAD and to propose interventions for identified risk factors. Diverging from previous research, this study utilized Pearson correlation analysis to investigate relationships between ABI measurements (ABI-R and ABI-L) and other variables, offering critical insight for clinicians in detecting asymmetrical vascular lesions.

Moreover, the research categorized participants into groups based on their 25(OH)D levels - deficient, insufficient, or sufficient - and analyzed PAD prevalence from the perspective of varying 25(OH)D levels. This stratification enhances the ability of healthcare providers to detect early signs of PAD.

Additionally, ROC analysis was conducted to assess the predictive accuracy of serum 25(OH)D levels in identifying PAD risk among patients with T2DM. This analysis provided a practical approach to evaluating the performance of serum indicators across different categories and determining the optimal threshold for classification. The ROC curves demonstrated the sensitivity and specificity of using serum 25(OH)D levels for PAD screening, aiming to enhance diagnostic accuracy and reduce the misdiagnosis rate.

This study, conducted as a single-center retrospective analysis, has inherent limitations. Consequently, the authors should pursue a multi-center, large-scale research initiative to delve deeper into the precise mechanisms underlying the influence of 25(OH)D on the development of PAD. For practical application, it is also imperative to incorporate real-world scenarios and establish a collaborative diagnostic model encompassing multiple indicators for a comprehensive assessment, thereby enhancing diagnostic accuracy. Moreover, to mitigate the impact of various confounding factors, future research should integrate NHANES or similar databases with Mendelian randomization techniques to unequivocally demonstrate the relationship and causality between serum 25(OH)D levels and PAD in T2DM patients.

In conclusion, this study holds significant theoretical and practical implications for assessing the risk of PAD occurrence and guiding clinical intervention. Broadly speaking, a low serum 25(OH)D level is associated with an elevated risk of PAD in T2DM patients. Routine clinical screening for 25(OH)D in T2DM patients can enhance the value of PAD detection, aid in identifying high-risk PAD populations, and

enable timely supplementation for patients with 25(OH)D deficiency.

Acknowledgements

It is supported by Shaoyang Science and Technology Plan Project (2022NS4154).

Disclosure of conflict of interest

None.

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