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Original Article

Association of serum vaspin levels with the presence and severity of peripheral arterial disease in type 2 diabetic patients

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Abstract: Objective: Vaspin, a newly discovered adipokine, is implicated to play an anti-atherosclerosis role. This study aims to determine the association of serum levels of vaspin with the presence and severity of peripheral arterial disease (PAD) in patients with type 2 diabetes mellitus (T2DM). Methods: This study consisted of 340 T2DM patients (160 without PAD and 180 with PAD). Serum levels of vaspin were evaluated using enzyme-linked immunosorbent assay method. Results: T2DM patients with PAD had significantly lower serum vaspin levels compared with those without PAD. Multivariable logistic regression analysis indicated that serum vaspin levels were inversely associated with the presence of PAD in T2DM patients (OR 0.002, 95% CI 0.000 to 0.014; $P<0.001$). Simple linear regression analysis showed that the serum levels of vaspin were negatively correlated with body mass index (BMI), triglycerides (TG), homeostasis model assessment of insulin resistance (HOMA-IR), and positively correlated with ankle-brachial index (ABI) in T2DM patients. Furthermore, only HOMA-IR and ABI remained associated with serum vaspin after multiple stepwise regression analysis. Conclusion: Decreased serum vaspin levels are associated with the presence and severity of PAD in T2DM patients.

Keywords: Vaspin, peripheral arterial disease, type 2 diabetes

Introduction

Peripheral arterial disease (PAD) is a common macrovascular complication in patients with type 2 diabetes mellitus (T2DM). Clinical study showed that diabetic patients had a 2.6-fold increased risk of developing PAD compared with people without diabetes, and this fold increased with the increase of duration of diabetes [1]. PAD is a common marker of atherosclerosis and acts as a predictor of morbidity and mortality in the case of cardiovascular and cerebrovascular diseases [2]. Smoking, advanced age, hypertension, dyslipidemia, degree and duration of hyperglycemia are considered to be important traditional risk factors for PAD [3].

Visceral adipose tissue-derived serine protease inhibitor (vaspin) has primarily been identified to be expressed in the visceral adipose of Otsuka Long-Evans Tokushima Fatty (OLETF) rats [4]. It improves glucose tolerance and enhances insulin sensitivity in mice [4]. Recently, vaspin was found to play an important protective role in the process of atherosclerosis. Vaspin could prevent free fatty acid-induced endothelial apoptosis [5]. In addition, serum vaspin levels were significantly reduced in patients with coronary artery disease than healthy controls [6]. Therefore, it is speculated that vaspin might be involved in the pathophysiology of PAD.

To our knowledge, the role of vaspin in the pathogenesis of PAD among T2DM patients has not been examined previously. The present study was designed to determine the association of serum levels of vaspin with the presence and severity of PAD in T2DM patients.

Materials and methods

Patients

340 patients diagnosed with T2DM were enrolled in this study. The diagnosis of T2DM was

Table 1. Clinical and biochemical characteristics of T2DM patients with and without PAD

	T2DM patients without PAD	T2DM patients with PAD	*P* value
N	160	180	
Age (years)	54.91±11.45	55.98±11.22	0.385
Gender (M/F)	84/76	87/93	0.443
Family history of T2DM n (%)	15 (9.38%)	20 (11.11%)	0.599
Duration of T2DM (years)	6.36±2.45	11.61±3.14	<0.001
BMI (Kg/m^2)	25.50±3.60	25.23±3.64	0.510
SBP (mmHg)	143.69±23.38	146.94±28.05	0.496
DBP (mmHg)	85.84±13.64	85.59±14.93	0.873
TC (mmol/L)	5.02±1.19	5.35±1.24	0.012
TG (mmol/L)	1.77±0.55	1.96±0.66	0.181
LDL-C (mmol/L)	3.25±0.88	3.68±1.20	<0.001
HDL-C (mmol/L)	1.31±0.36	1.26±0.31	0.122
FPG (mmol/L)	8.92±3.58	9.04±3.50	0.745
P_{2h}PG (mmol/L)	14.83±2.66	14.56±2.70	0.351
HbA1c (%)	9.59±2.41	9.52±2.45	0.781
HOMA-IR	3.63±0.70	4.37±0.89	0.002
Vaspin (ng/mL)	0.76 (0.63-0.92)	0.55 (0.39-0.66)	<0.001
ABI	1.17±0.15	0.68±0.12	<0.001

T2DM, type 2 diabetes mellitus; PAD, peripheral arterial disease; BMI, body mass index; 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; FPG, fasting plasma glucose; P_{2h}PG, 2-hour postprandial plasma glucose; HOMA-IR, homeostasis model assessment of insulin resistance; ABI, ankle-brachial index.

made according to the criteria using a fasting glucose level ≥7.0 mmol/L or 2-hour postprandial plasma glucose (P_{2h}PG) level ≥11.1 mmol/L. Patients were excluded if they had malignancy, renal impairment, type 1 diabetes, pregnancy, previous diagnosis of osteoporosis, macrovascular event, a recent or current fracture or foot ulcer/osteomyelitis. The diagnoses of PAD were confirmed by color Doppler ultrasonography with an ankle-brachial index (ABI) <0.9 on either side of the lower extremities. Patients with ABI ≥1.4 in at least one limb were diagnosed as medial arterial calcification (MAC) and were excluded from the current analysis. The lower of the two ABI was used for further analysis.

This study was planned according to the ethics guidelines of the Helsinki Declaration and was approved by the Institutional Research Ethics Board of our hospital. All patients gave written informed consent regarding participation in this study.

Measurements

Anthropometric (height, weight and blood pressures), clinical and laboratory analysis were performed. Venous blood was collected after a minimum of 10 hours of absolute diet. Serum vaspin was determined using an enzyme-linked immunosorbent assay (ALPCO Diagnostic, Salem NH, USA).

Statistical analysis

Statistical analysis was carried out using SPSS version 13.0 software program (SPSS Inc, Chicago, Illinois). The results were expressed as means ± standard errors (interquartile range). Comparison of the characteristics between T2DM patients with and without PAD was performed by unpaired t test, Chi-square tests, or Mann-Whitney U test. Univariate analysis was performed and the variables with a *P*<0.10 were then entered into a backward stepwise multivariate logistic regression model to calculate the Odds ratio values (OR) and 95% confidence intervals (CI) for the presence of PAD in T2DM patients. The correlation between serum vaspin and other parameters were analyzed using simple linear regression analysis. Then a multiple stepwise linear regression analysis was used to determine the contribution of various factors to serum vaspin. *P* values less than 0.05 were considered to be statistically significant.

Results

Baseline clinical characteristics

The clinical and laboratory characteristics of T2DM patients with and without PAD are presented in **Table 1**. T2DM patients with PAD showed higher levels of duration of T2DM, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), homeostasis model assessment of insulin resistance (HOMA-IR), and decreased ABI compared with those without PAD. There were no significant differences in other characteristics between the two groups.

Table 2. Logistic regression Analysis for the presence of PAD in T2DM patients

	Simple regression		Multiple regression	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Age (years)	1.008 (0.990-1.028)	0.384		
Gender (M/F)	1.181 (0.771-1.810)	0.443		
Family history of T2DM	1.208 (0.596-2.448)	0.599		
Duration of T2DM (years)	2.113 (1.798-2.482)	<0.001	2.320 (1.880-2.863)	<0.001
BMI (Kg/m^2)	0.980 (0.924-1.039)	0.495		
SBP (mmHg)	1.005 (0.997-1.013)	0.249		
DBP (mmHg)	0.999 (0.984-1.014)	0.872		
TC (mmol/L)	1.258 (1.048-1.510)	0.014	1.157 (0.752-1.780)	0.508
TG (mmol/L)	1.121 (0.947-1.328)	0.184		
LDL-C (mmol/L)	1.487 (1.192-1.856)	<0.001	1.429 (0.857-2.380)	0.171
HDL-C (mmol/L)	0.602 (0.316-1.148)	0.123		
FPG (mmol/L)	1.010 (0.951-1.073)	0.744		
P2PG (mmol/L)	0.963 (0.889-1.043)	0.350		
HbA1c (%)	0.988 (0.905-1.078)	0.780		
HOMA-IR	1.245 (1.080-1.434)	0.002	1.399 (1.095-1.789)	0.007
Vaspin (ng/mL)	0.003 (0.001-0.012)	<0.001	0.002 (0.000-0.014)	<0.001

T2DM, type 2 diabetes mellitus; PAD, peripheral arterial disease; BMI, body mass index; 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; FPG, fasting plasma glucose; P_{2h}PG, 2-hour postprandial plasma glucose; HOMA-IR, homeostasis model assessment of insulin resistance; ABI, ankle-brachial index.

Table 3. Linear regression analyses between serum vaspin and other clinical parameters

Parameters	R	*P* value
Age (years)	0.023	0.669
Gender (M/F)	0.077	0.155
Family history of T2DM	0.030	0.576
Duration of T2DM (years)	-0.067	0.216
BMI (Kg/m^2)	-0.128	0.019
SBP (mmHg)	-0.082	0.131
DBP (mmHg)	-0.070	0.197
TC (mmol/L)	-0.047	0.389
TG (mmol/L)	-0.153	0.005
LDL-C (mmol/L)	-0.088	0.106
HDL-C (mmol/L)	0.063	0.245
FPG (mmol/L)	-0.034	0.537
P2PG (mmol/L)	-0.061	0.261
HbA1c (%)	-0.043	0.432
HOMA-IR	-0.235	<0.001
ABI	0.186	0.001

T2DM, type 2 diabetes mellitus; PAD, peripheral arterial disease; BMI, body mass index; 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; FPG, fasting plasma glucose; P_{2h}PG, 2-hour postprandial plasma glucose; HOMA-IR, homeostasis model assessment of insulin resistance; ABI, ankle-brachial index.

Association of serum vaspin levels with the presence of PAD in T2DM patients

The results indicated serum vaspin levels were significantly decreased in T2DM patients with PAD compared with those without PAD (**Table 1**). Simple logistic regression analysis showed that duration of T2DM (OR 2.113, 95% CI 1.798 to 2.482; *P*<0.001), TC (OR 1.258, 95% CI 1.048 to 1.510; *P*=0.014), LDL-C (OR 1.487, 95% CI 1.192 to 1.856; *P*<0.001), HOMA-IR (OR 1.245, 95% CI 1.080 to 1.434; *P*=0.002), and serum vaspin (OR 0.003, 95% CI 0.001 to 0.012; *P*<0.001) showed relative significant correlations with the presence of PAD in T2DM patients (**Table 2**). All these variables were then entered into a backward stepwise multivariate logistic regression model. Multivariate logistic regression of revealed that serum vaspin levels remained as a significant and independent predictor of T2DM (OR 0.002, 95% CI 0.000 to 0.014; *P*<0.001) (**Table 2**).

Association of serum vaspin levels with other clinical characteristics

Simple regression analyses showed that serum vaspin levels in T2DM patients were negatively

correlated with BMI (r=-0.128, *P*=0.019), triglycerides (TG) (r=-0.153, *P*=0.005), HOMA-IR (r=-0.235, *P*<0.001), and positively correlated with ABI (r=0.186, *P*=0.001) (**Table 3**). Then variables including BMI, TG, HOMA-IR, and ABI were incorporated into the stepwise linear regression model. Multiple stepwise regression analysis shows that only HOMA-IR (β=-0.216, *P*<0.001) and ABI (β=0.168, *P*=0.005) remained associated with serum vaspin.

Discussion

In the present study, we found that T2DM patients with PAD had significantly lower serum vaspin levels compared with those without PAD. In addition, serum vaspin levels were negatively correlated with BMI, TG, HOMA-IR, and positively correlated with ABI. To the best of our knowledge, this is the first cross-sectional study that demonstrates the association of serum vaspin levels with the presence and severity of PAD in T2DM patients.

Nowadays, biomarkers are used in early disease diagnosis, prognosis of disease progress, and evaluating therapeutic effects of many diseases. Adipose tissue is an active metabolic and endocrine organ. Adipocytes produce several substances, so called as adipokines, which influence glucose and lipid metabolism. These adipokines includes tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), leptin, resistin, visfatin, and chemerin and so on [7]. These adipokines play important roles in many disease such as obesity, metabolic syndrome, diabetes, and cardiovascular disease [8]. The present study revealed that T2DM patients with PAD had significantly lower serum vaspin levels compared with those without PAD, which suggesting the potential role of vaspin in the pathophysiology of PAD in diabetes. Similar results were found in another study, which reported that serum vaspin levels were significantly lower in T2DM patients with carotid plaque than in those without [9]. Furthermore, the current results showed that serum levels of vaspin were positively correlated with ABI. Serum vaspin levels are correlated with the severity of PAD. Therefore, serum vaspin levels are suggested to be an independent predicting biomarker of the presence and severity of PAD in T2DM patients.

Vaspin plays an important role in the process of atherosclerosis. Vaspin increased endothelial nitric oxide synthase activity by reducing asymmetric dimethylarginine level [10]. Vaspin inhibited high glucose-induced vascular smooth muscle cells (VSMC) proliferation and chemokinesis by preventing reactive oxygen species (ROS) activation and MAPK, PI3K/Akt, and NF-κB signaling [11]. In addition, vaspin was found to inhibit platelet-derived growth factor-BB-induced VSMC migration via preventing the ROS generation [12]. All these results indicate that vaspin play as an anti-atherogenic actor. Serum levels of vaspin were also found to be significantly lower in patients with coronary artery disease [13] and carotid stenosis [14], which point to the anti-atherogenic role of vaspin.

The limitation of the present study should be considered. First, the modest sample size in this study could impede our discovery of less-strong associations; larger sample size is required to determine whether the significant results were false positive. Second, this study was cross-sectional. A prospective cohort study may provide more definitive evidence about the association of serum vaspin with the presence and severity of PAD in T2DM patients.

In conclusion, this study showed that T2DM patients with PAD had significantly lower serum vaspin levels compared with those without PAD. Serum levels of vaspin were significantly correlated with ABI. Decreased serum vaspin levels are suggested to be an independent predicting marker of the presence and severity of PAD in T2DM patients.

Disclosure of conflict of interest

None.

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