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Serum Nesfatin-1 is Reduced in Type 2 Diabetes Mellitus Patients with Peripheral Arterial Disease

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Background: Nesfatin-1, recently identified as a satiety regulator, elicits an anti-atherosclerosis effect. Our study was designed to determine whether there is an association between serum nesfatin-1 and the development and severity of peripheral arterial disease (PAD) in patients with type 2 diabetes mellitus (T2DM).

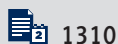
Material/Methods: This cross-sectional study included 355 T2DM patients (200 without PAD and 155 with PAD).

Results: T2DM patients with PAD exhibited marked lower serum nesfatin-1 concentrations than those without PAD. Multivariable logistic regression analysis indicated an inverse association of serum nesfatin-1 concentrations with the development of PAD in T2DM patients (OR 0.008, 95% CI 0.002 to 0.028; $P < 0.001$). Simple linear regression analysis showed a marked correlation between serum nesfatin-1 concentrations and body mass index (BMI), homeostasis model assessment of insulin resistance (HOMA-IR), C-reactive protein (CRP), and ankle-brachial index (ABI) in T2DM patients. By contrast, multivariable analysis showed only BMI and ABI as independent correlates of serum nesfatin-1.

Conclusions: Our study shows an association of serum nesfatin-1 concentrations and the development and severity of PAD in T2DM patients.

MeSH Keywords: **Adipokines • Diabetes Mellitus, Type 2 • Peripheral Arterial Disease**

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Background

Peripheral arterial disease (PAD) is a clinical atherosclerotic arterial occlusive disease that affects the lower extremities. Diabetes, together with smoking, age, and fibrinogen, are traditional risk factors for PAD [1]. In addition, genetic factors are also involved in the mechanism of PAD or atherosclerosis [2]. Atherosclerosis is a complex disease with various intermediate phenotypes; this disease is also influenced by many factors [3]. Candidate gene polymorphisms related to carotid intima-media thickness (CIMT), lipid and glucose metabolism, inflammation, and statin metabolism are demonstrated to be correlated with the occurrence and progression of PAD or atherosclerosis [4]. The relative risk of PAD in subjects with type 2 diabetes mellitus (T2DM) is 3–4 times higher than that of normal subjects [5]. The occurrence of PAD leads to an increased mortality because of cardiovascular and cerebrovascular diseases and a poor quality of life as a result of leg pain, gangrene, and amputation [6]. Hence, the risk for PAD should be predicted at a relatively early stage and effective strategies should also be implemented to delay or prevent PAD development.

Nesfatin-1 is a newly discovered peptide with 82-amino acids [7]. In rats, intracerebroventricular injection of nesfatin-1 causes inhibited appetite and then weight loss, while

the antibody-neutralizing nesfatin-1 treatment results in increased food intake [7]. Recently, nesfatin-1 was found to exert cardiovascular actions in the brain by increasing sympathetic nerve activity, thereby increasing mean arterial pressure [8]. Furthermore, subjects with acute myocardial infarction had relatively lower serum nesfatin-1 concentrations than healthy volunteers [9]. This finding indicates that nesfatin-1 may play a role in atherosclerosis. Thus, nesfatin-1 is hypothesized to contribute to PAD development in T2DM.

The present investigation was designed to assess the association between serum nesfatin-1 and the development and severity of peripheral arterial disease (PAD) in patients with T2DM.

Material and Methods

Patients

The study population consisted of 355 consecutive T2DM patients. The diagnosis of T2DM was made according to fasting glucose level ≥ 7.0 mmol/L or 2-h postprandial plasma glucose level ≥ 11.1 mmol/L [10]. Patients who had a clinical history of T2DM and were receiving oral hypoglycemic or parenteral insulin medications were also eligible for this study. Exclusion

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

	T2DM patients without PAD	T2DM patients with PAD	P value
N	200	155	
Age (years)	57.38 \pm 10.71	57.82 \pm 9.91	0.696
Gender (M/F)	95/105	70/85	0.661
Family history of T2DM n (%)	10 (5.00%)	7 (4.52%)	0.832
Duration of T2DM (years)	8.58 \pm 3.43	11.39 \pm 2.86	<0.001
BMI (Kg/m ²)	25.41 \pm 3.84	25.14 \pm 3.72	0.510
SBP (mmHg)	144.62 \pm 25.91	148.48 \pm 22.97	0.145
DBP (mmHg)	86.21 \pm 14.54	84.87 \pm 13.54	0.376
TC (mmol/L)	5.14 \pm 1.15	5.42 \pm 1.27	0.031
TG (mmol/L)	1.87 \pm 0.60	1.92 \pm 0.59	0.783
LDL-C (mmol/L)	3.34 \pm 0.99	3.59 \pm 1.02	0.016
HDL-C (mmol/L)	1.28 \pm 0.34	1.28 \pm 0.28	0.946
FPG (mmol/L)	8.94 \pm 3.50	8.94 \pm 3.23	0.997
P2PG (mmol/L)	15.66 \pm 3.47	15.33 \pm 3.60	0.378
HbA1c (%)	9.34 \pm 2.24	9.20 \pm 2.13	0.563
HOMA-IR	3.63 \pm 0.70	4.37 \pm 0.89	<0.001
CRP (mg/L)	2.35 (1.40–3.40)	2.50 (1.50–4.40)	0.108
Nesfatin-1 (ng/mL)	1.20 (0.96–1.36)	0.91 (0.70–1.12)	<0.001
ABI	1.16 \pm 0.14	0.71 \pm 0.13	<0.001

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)	1.004 (0.984–1.025)	0.695		
Gender (M/F)	1.099 (0.721–1.673)	0.661		
Family history of T2DM	0.899 (0.334–2.417)	0.832		
Duration of T2DM (years)	1.313 (1.216–1.417)	<0.001	1.312 (1.202–1.433)	<0.001
BMI (Kg/m ²)	0.981 (0.928–1.038)	0.509		
SBP (mmHg)	1.006 (0.998–1.015)	0.145		
DBP (mmHg)	0.993 (0.978–1.008)	0.376		
TC (mmol/L)	1.212 (1.016–1.447)	0.033	1.186 (0.656–2.144)	0.572
TG (mmol/L)	1.020 (0.888–1.171)	0.782		
LDL-C (mmol/L)	1.294 (1.046–1.600)	0.018	1.106 (0.552–2.219)	0.776
HDL-C (mmol/L)	1.023 (0.522–2.005)	0.946		
FPG (mmol/L)	1.000 (0.940–1.064)	0.997		
P2PG (mmol/L)	0.973 (0.917–1.033)	0.377		
HbA1c (%)	0.972 (0.883–1.070)	0.561		
HOMA-IR	1.229 (1.092–1.383)	<0.001	1.296 (1.108–1.516)	0.001
CRP (mg/L)	1.160 (1.036–1.299)	0.009	1.119 (0.967–1.294)	0.131
Nesfatin-1 (ng/mL)	0.010 (0.003–0.029)	<0.001	0.008 (0.002–0.028)	<0.001

criteria were critical illness within the last 6 months, cancer, renal failure, connective tissue diseases, and hormone replacement therapy. Diagnosis of PAD was based on an ankle-brachial index (ABI) <0.9 on either leg. Patients with ABI ≥1.4 in at least 1 limb were expected to have non-compressible arteries and were excluded. The lower of the 2 ABIs was used for further analysis.

The study protocol was approved by the Research Ethics Committee of our hospital, and all patients gave their informed consent before study commencement.

Measurements

The quantitative determination of nesfatin-1 levels was done using a commercial enzyme-linked immunosorbent assay kit (Phoenix Pharmaceuticals, Inc., USA).

Statistical analysis

Data are displayed as median (25th percentile, 75th percentile) or mean ± standard deviation. Differences between T2DM patients with and without PAD were compared using unpaired t

test, chi-square tests, or Mann-Whitney U test. Univariate analysis was performed to examine the independent predictors for PAD in T2DM patients. Data were analyzed utilizing univariate linear regression models looking for marked associations between serum nesfatin-1 and other parameters. Statistical analysis was carried out using SPSS version 13.0 software program (SPSS Inc, Chicago, Illinois). We regarded a P value of less than 0.05 as statistically meaningful.

Results

Baseline clinical characteristics

As shown in Table 1, T2DM patients with PAD presented significantly longer T2DM duration, and higher total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), HOMA-IR, and ABI than those without PAD.

Correlation of serum nesfatin-1 levels with PAD

As shown in Table 1, serum nesfatin-1 was markedly lowered in T2DM patients with PAD compared with those without PAD.

Table 3. Linear regression analyses between serum nesfatin-1 and other clinical parameters.

Parameters	r	P value
Age (years)	0.042	0.435
Gender (M/F)	0.077	0.148
Family history of T2DM	0.054	0.307
Duration of T2DM (years)	-0.082	0.125
BMI (Kg/m ²)	-0.218	<0.001
SBP (mmHg)	0.051	0.339
DBP (mmHg)	0.057	0.286
TC (mmol/L)	-0.063	0.234
TG (mmol/L)	-0.041	0.440
LDL-C (mmol/L)	-0.085	0.112
HDL-C (mmol/L)	0.079	0.135
FPG (mmol/L)	-0.050	0.344
P2PG (mmol/L)	-0.028	0.600
HbA1c (%)	-0.038	0.473
HOMA-IR	-0.178	0.001
CRP (mg/L)	-0.149	0.005
ABI	0.295	<0.001

Simple logistic regression analysis indicated that T2DM duration, TC, LDL-C, HOMA-IR, CRP, and serum nesfatin-1 were associated with PAD (Table 2). Multivariable logistic regression analysis identified HOMA-IR and serum nesfatin-1 as independent correlates of PAD in T2DM patients (Table 2).

Correlation between serum nesfatin-1 and clinical parameters

Simple linear regression analysis revealed a significantly negative correlation between serum nesfatin-1 and BMI, HOMA-IR, and CRP, and a significantly positive correlation with ABI. However, multivariable analysis showed only BMI and ABI as independent correlates of serum nesfatin-1 (Table 3).

Discussion

PAD is a chronic occlusive disease that occurs in the lower extremities. Pain in leg muscles during walking, a condition known as intermittent claudication, is one of the most common clinical presentation of PAD [11]. PAD can result in chronic pain in the legs and eventually non-healing wounds, gangrene, and limb loss [12]. PAD is a common marker of atherosclerosis and acts as a predictor of morbidity and mortality due to cardiovascular and cerebrovascular diseases [13]. PAD patients show

significantly lower health-related quality of life than the general population, mainly because of functional limitations caused by claudication [14]. In addition, PAD frequency is higher in T2DM subjects than in healthy subjects [5]. We found lower serum nesfatin-1 concentrations in T2DM patients with PAD than those without PAD. There is also a marked correlation of serum nesfatin-1 with ABI. These results indicate the contributory role of nesfatin-1 in PAD pathogenesis.

Atherosclerosis and thrombosis contribute to acute myocardial infarction (AMI) development. Inflammation, neovascularization, apoptosis, and hypercoagulable state, as well as growth factors, are considered to be involved in the mechanisms of atherosclerosis and AMI [15]. Serum levels of nesfatin-1 were reduced in patients with AMI [9], demonstrating the anti-atherogenic effects of nesfatin-1. In addition, nesfatin-1 plays a key role in anti-inflammation. Inflammation is closely correlated with atherosclerosis development [16]. Nesfatin-1 administration after head trauma suppressed gene expressions of nuclear factor kappa-B and reduced tumor necrosis factor-alpha, interleukin-1 β , and interleukin-6 concentrations in rat traumatic brain tissues [17]. Dai et al. reported an inverse association of plasma nesfatin-1 with high-sensitivity CRP [9]. Likewise, we found an association between serum nesfatin-1 levels and CRP. Therefore, nesfatin-1 may inversely contribute to atherosclerosis and PAD through eliciting anti-inflammatory effects.

Yosten demonstrated that intracerebroventricular administration of nesfatin-1 increased arterial blood pressure of rats, presumably via the activation of sympathetic nerves [8]. In addition, pretreatment of oxytocin receptor antagonist on conscious and unrestrained male rats reversed the effect of nesfatin-1 on both food and water intake and on mean arterial pressure hypertension [18]. Plasma nesfatin-1 concentrations were significantly elevated in hypertensive patients [19] and plasma nesfatin-1 levels were positively correlated with SBP and DBP [19]. This indicates the blood pressure regulatory effects of nesfatin-1. However, no correlation was found between serum nesfatin-1 and blood pressure. Further basic studies should be conducted to explain the exact function of nesfatin-1 in blood pressure regulation.

We observed that serum nesfatin-1 concentrations were negatively associated with BMI and HOMA-IR. Serum levels of nesfatin-1 were negatively associated with BMI and HOMA-IR in non-obese male patients [20] and in polycystic ovary syndrome patients [21]. Obesity and insulin resistance are multiple compounding factors of metabolic syndrome (MetS), indicating that nesfatin-1 may be involved in the mechanism of obesity and insulin resistance, which are

factors of metabolic syndrome (MetS). MetS is closely associated with PAD. It is reported that PAD prevalence in subjects with MetS was 7.0% compared with 3.3% in subjects without MetS [22]. Thus, nesfatin-1 possibly mediates the interplay between MetS and PAD.

This study has several potential limitations. First, a cross-sectional design was used in this study. A prospective cohort study or a randomized case control study could provide more definitive evidence. Second, baseline characteristics between T2DM populations with and without PAD were not comparable, which may have had an effect on the conclusion. Third, other microvascular or macrovascular complications of T2DM were not investigated.

Conclusions

Serum nesfatin-1 levels were inversely correlated with the development and severity of PAD in T2DM patients. Serum nesfatin-1 may be utilized as a predicting biomarker for PAD disease risk. Further investigations are needed to examine the exact function of nesfatin-1 in PAD pathogenesis.

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