Positive correlation of serum leptin levels and peripheral arterial stiffness in patients with type 2 diabetes

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INTRODUCTION

The arterial system undergoes structural and functional changes with age, characterized by endothelial dysfunction, wall thickening, reduced distensibility, and arterial stiffening [1]. Arterial stiffness represents vascular damage and has been identified as an independent risk factor for the development of cardiovascular mortality [2]. Arterial stiffness can be measured by pulse wave velocity (PWV). This method is noninvasive and reproducible and is the simplest way to measure arterial stiffness [3]. Brachial-ankle PWV (baPWV) is one measure arterial stiffness using brachial to ankle arterial wave analyses and has been used to assess peripheral arterial stiffness (PAS) compared with carotid-femoral PWV which is used to assess central arterial stiffness using carotid-to-femoral arterial waves [3,4]. A meta-analysis of 12 cohort studies confirmed that baPWV is an independent predictor for cardiovascular disease onset, and an increase of 1 m/s was associated with a 12% increase in the risk of cardiovascular events [5]. baPWV is also a useful early marker for the detection of vascular dysfunction in patients with type 2 diabetes mellitus (DM) [6].

Leptin, a 16-kDa adipokine hormone peptide encoded by the obesity (ob) gene, regulates energy balance by inhibiting hunger [7]. Leptin exerts many potentially atherogenic effects such as induction of endothelial dysfunction and stimulation of the inflammatory reaction, oxidative stress, platelet aggregation, and vascular smooth muscle hypertrophy [8]. Circulating leptin is considered a risk factor for coronary heart disease [9], and a predictor of central arterial stiffness in patients with coronary artery disease [10], hypertension [11], and kidney transplantation [12]. Moreover,

ABSTRACT

Objective: Leptin plays a pathophysiologic role in the pathogenesis of aortic dysfunction and peripheral arterial stiffness (PAS). Our aim was to evaluate the risk factors for developing PAS and the association of leptin and PAS in patients with type 2 diabetes mellitus (DM). Materials and Methods: Fasting blood samples were obtained for biochemical data and leptin determinations from 105 patients with type 2 DM. In this study, we applied an automatic pulse wave analyzer (VaSera VS-1000) to measure the brachial-ankle pulse wave velocity (baPWV); a baPWV value \(>14.0\) m/s on either side was considered high PAS. Results: Seventy-five patients (71.4%) had high PAS and they included a higher percentage of patients with hypertension \((P < 0.001)\), older age \((P < 0.001)\), and a higher body fat mass \((P = 0.043)\), systolic blood pressure \((P < 0.001)\), diastolic blood pressure \((P = 0.016)\), serum blood urea nitrogen \((P = 0.003)\), and leptin level \((P < 0.001)\), and lower height \((P = 0.027)\) and glomerular filtration rate \((P < 0.001)\) compared with type 2 DM patients with low PAS. After adjusting for factors significantly associated with PAS in these patients by multivariate logistic regression analysis, age \((β = 0.470, \text{adjusted } R^2 \text{ change} = 0.279; \ P < 0.001)\), logarithmically transformed leptin \((\text{log-leptin}, β = 0.259, \text{adjusted } R^2 \text{ change} = 0.085; \ P = 0.001)\), and hypertension \((β = 0.197, \text{adjusted } R^2 \text{ change} = 0.031; \ P = 0.011)\) were significant independent predictors of PAS in type 2 DM patients. Conclusion: The serum leptin level could be a predictor of PAS in type 2 DM patients.

KEYWORDS: Brachial-ankle pulse wave velocity, Leptin, Peripheral arterial stiffness, Type 2 diabetes mellitus
the serum leptin level is also a predictor of PAS in kidney transplantation patients [13]. baPWV was a predictor of all-cause mortality in diabetic patients after nontraumatic lower extremity amputation [14]. The aim of the present study was to determine the risk factors for PAS and the relationship between the serum leptin concentration and PAS in type 2 DM patients.

MATERIALS AND METHODS

Participants

We enrolled 105 patients with type 2 DM from a medical center in Hualien, Taiwan from November 2014 to March 2015. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Protection of the Human Subjects Institutional Review Board of Tzu Chi University and Hospital (IRB103-136-A). Informed written consent was obtained from all participants before their enrolment in this study. The study participants were recruited in the outpatient metabolism and endocrinology clinic at Buddhist Tzu Chi General Hospital, Hualien, Taiwan. Patients were excluded if they had an acute infection, limb amputation, acute myocardial infarction, heart failure, or malignancy at the time of blood sampling, or if they refused to provide informed consent for the study.

Anthropometric analysis

Body weight of the participant was measured in light clothing and without shoes to the nearest 0.5 kg, and body height was measured to the nearest 0.5 cm. Waist circumference was measured using a tape measure around the waist from the point between the lowest ribs and the hip bones with the hands on the hips. Body mass index (BMI) was calculated as the weight in kilograms divided by the height in meters squared. Bioimpedance measurements of fat mass were performed at the bedside according to the standard tetrapolar whole-body (hand-foot) technique, using a single-frequency (50 kHz) analyzer (Biodynamic-450, Biodynamics Corporation, Seattle, USA) [10-13,15,16]. All measurements were carried out by the same operator.

Biochemical investigations

Fasting blood samples (approximately 5 mL) were immediately centrifuged at 3000 × g for 10 min. Serum levels of albumin, blood urea nitrogen (BUN), creatinine (Cre), fasting glucose, glycated hemoglobin, total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol were measured using an autoanalyzer (Siemens Advia 1800, Siemens Healthcare GmbH, Henkestr, Germany) [10-13,15,16]. Serum leptin (SPI-BIO, Montigny le Bretonneux, France) concentrations were determined using a commercially available enzyme immunoassay [10-13,15]. The estimated glomerular filtration rate (GFR) was calculated using the Modification of Diet in Renal Disease equation in this study.

Measurements of blood pressure and brachial-ankle pulse wave velocity

After blood sampling, patients rested in the supine position for 10 min. Blood pressure was determined by an automatic upper arm oscillometric device, and systolic blood pressure (SBP) and diastolic blood pressure (DBP) were checked three times in the right brachial artery. Hypertension was defined as SBP ≥140 mmHg, and/or DBP ≥90 mmHg, or if the patient had received any antihypertensive medication in the past 2 weeks. Then, four pneumatic cuffs connected to both plethysmographic and oscillometric sensors were wrapped around both upper arms and ankles for the assessment of the baPWV by a volume-plethysmographic apparatus (VaSera VS-1000, Fukuda Denki Co. Ltd., Tokyo, Japan) [13,16]. To monitor the electrocardiogram and heart sounds, a microphone was placed on the left side of the sternum, and electrocardiogram electrodes were placed on both wrists. Phonocardiography, electrography, and arterial pressure waveforms of the brachial and ankle arteries were measured, and the left-baPWV and right-baPWV were automatically calculated by the analyzer. The baPWV was calculated as the length of an arterial segment between the brachium and ankle divided by the time interval between the wavefront of the brachial waveform and that of the ankle waveform. Based on the recursive partitioning analysis in the Kyushu Prevention Study of Atherosclerosis, a baPWV of 14 m/s was considered a statistically adequate cutoff point for coronary artery events and cerebrovascular events in diabetic populations [17]. Therefore, in the present study, patients were in the high PAS group if their left or right baPWV values were >14.0 m/s [13,16,18].

Statistical analysis

Data were tested for normal distribution using the Kolmogorov–Smirnov test. Data were expressed as means ± standard deviation (SD) for normally distributed data and comparisons between patients were performed using the Student’s independent t-test (two-tailed). Data were expressed as medians and interquartile ranges for nonnormally distributed data (TG, fasting glucose, BUN, Cre, and leptin) and comparisons between patients were performed using the Mann–Whitney U-test. Data expressed as the number and percentage of patients were analyzed by the Chi-square test. TG, fasting glucose, BUN, Cre, and leptin were not normally distributed and underwent base 10 logarithmic transformations to achieve normality. Variables that were significantly associated with PAS in type 2 DM patients were tested for independence in multivariate forward stepwise regression analysis (adopted factors: age, hypertension, height, body fat mass, SBP, DBP, log-BUN, GFR, and log-leptin). Data were analyzed using SPSS for Windows (version 19.0; SPSS Inc., Chicago, IL, USA). The P < 0.05 was considered statistically significant.

RESULTS

The clinical characteristics and biochemical data of our 105 type 2 DM patients are presented in Table 1. Seventy-five patients (71.4%) were in the high PAS group with a left or right baPWV value >14.0 m/s, and the others were classified as the low PAS group. Compared with the low PAS group, type 2 DM patients in the high PAS group were older (P < 0.001) and had higher body fat mass (P = 0.043), SBP (P < 0.001), DBP (P = 0.016), BUN (P = 0.003), and leptin levels (P < 0.001), and lower height (P = 0.027) and GFR (P < 0.001). Comparisons of age and leptin levels in the high and low PAS groups in patients with type 2 DM are presented in Figure 1.
The data for gender, hypertension, and drugs use are presented in Table 2. Among these patients, 59 had hypertension (56.2%). Type 2 DM patients with hypertension ($P < 0.001$) were prone to develop high PAS. No statistically significant differences in PASs were found in patients using statins, fibrates, or antidiabetic drugs.

Multivariable forward stepwise linear regression analysis was used to delineate the significant variables associated with PAS in type 2 DM patients, and the results are presented in Table 3. We found that age ($\beta = 0.470$, adjusted $R^2$ change = 0.279; $P < 0.001$), logarithmically transformed leptin (log-leptin, $\beta = 0.259$, adjusted $R^2$ change = 0.085; $P = 0.001$), and hypertension ($\beta = 0.197$, adjusted $R^2$ change = 0.031; $P = 0.011$) were independent predictors of PAS in type 2 DM patients.

**Discussion**

The results of our study showed a high prevalence of PAS in patients with type 2 DM. The fasting serum leptin level, hypertension, and older age were independent predictors of PAS among the type 2 DM patients.

Arterial stiffness is related to atherosclerosis and cardiovascular disease and has been considered a strong independent predictor of coronary events and cardiovascular mortality [2]. Katakami et al. noted the cumulative incidence rates of cardiovascular events (coronary heart disease, cerebrovascular events, peripheral artery disease, and heart failure) were significantly higher in patients with high baPWV values ($\geq 1550$ cm/s) than those with low baPWV values ($<1550$ cm/s), and a multivariate analysis in 1040 type 2 DM patients revealed that baPWV was still an independent predictor for cardiovascular events even after...
Table 2: Distribution of diabetic patients with high and low peripheral arterial stiffness in subgroup analysis

| Characteristics | Low PAS group (%) | High PAS group (%) | P   |
|-----------------|-------------------|-------------------|-----|
| Gender          |                   |                   |     |
| Male            | 17 (56.7)         | 34 (45.3)         | 0.294 |
| Female          | 13 (43.3)         | 41 (54.7)         |     |
| Hypertension    |                   |                   |     |
| No              | 22 (73.3)         | 24 (32.0)         | <0.001* |
| Yes             | 8 (26.7)          | 51 (68.0)         |     |
| Statins         |                   |                   |     |
| No              | 16 (53.3)         | 38 (50.7)         | 0.805 |
| Yes             | 14 (46.7)         | 37 (49.3)         |     |
| Fibrates        |                   |                   |     |
| No              | 29 (96.7)         | 71 (94.7)         | 0.664 |
| Yes             | 1 (3.3)           | 4 (5.3)           |     |
| Metformin       |                   |                   |     |
| No              | 17 (56.7)         | 30 (40.0)         | 0.121 |
| Yes             | 13 (43.3)         | 45 (60.0)         |     |
| Sulfonyluricas  |                   |                   |     |
| No              | 13 (43.3)         | 36 (48.0)         | 0.665 |
| Yes             | 17 (56.7)         | 39 (52.0)         |     |
| DDP-4 inhibitors|                   |                   |     |
| No              | 9 (30.0)          | 34 (45.3)         | 0.149 |
| Yes             | 21 (70.0)         | 41 (54.7)         |     |
| Thiazolidinediones|               |                   |     |
| No              | 30 (100.0)        | 74 (98.7)         | 0.525 |
| Yes             | 0                 | 1 (1.3)           |     |
| Insulin         |                   |                   |     |
| No              | 26 (86.7)         | 54 (72.0)         | 0.111 |
| Yes             | 4 (13.3)          | 21 (28.0)         |     |

*P < 0.05 was considered statistically significant. Data are expressed as n (%) of patients. Analysis was done using the Chi-square test. PAS: Peripheral arterial stiffness, DDP-4: Dipeptidyl peptidase 4

Table 3: Multivariable stepwise linear regression analysis of peripheral artery stiffness in 105 diabetic patients

| Items                  | β      | Adjusted R² | Adjusted R² change | P   |
|------------------------|--------|-------------|--------------------|-----|
| Age (years)            | 0.470  | 0.279       | 0.279              | <0.001* |
| Log-leptin (ng/mL)     | 0.259  | 0.364       | 0.085              | 0.001* |
| Hypertension           | 0.197  | 0.395       | 0.031              | 0.011* |

Data for BUN and leptin levels showed skewed distributions and therefore were log-transformed before analysis. *P<0.05 was considered statistically significant in multivariable stepwise linear regression analysis (adopted factors: Age, hypertension, height, body fat mass, SBP, DBP, log-BUN, GFR, and log-leptin). BUN: Blood urea nitrogen, GFR: Glomerular filtration rate, DBP: Diastolic blood pressure, SBP: Systolic blood pressure

There were some limitations in this current study. First, this was an observational, single-center study with a small number of participants with type 2 DM. Second, participants in this study were not differentiated by race, limiting the conclusions of our findings in other ethnicities. Third, although we collected data on antidiabetic and anti-lipid drugs, we did not collect data on medications such as antihypertensives which could have potentially affected serum leptin levels or baPWV values. Another limitation is that the blood pressure was measured with the patient in a supine position, which may have affected the reading. Usually, blood pressure is significantly lower in the sitting than the supine position [32]. Whether a patient’s blood pressure is measured in the sitting or supine position may impact the results.
position affects baPWV values is unclear. Further long-term prospective studies are needed to follow-up on the trend between serum leptin and PAS in type 2 DM patients.

**CONCLUSION**

The present study revealed a high prevalence of PAS in type 2 DM patients. Hyperleptinemia positively correlated with PAS in type 2 DM patients.

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**Conflicts of interest**

There are no conflicts of interest.

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