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Relationship between Serum Pre-B Cell Colony-Enhancing Factor/Visfatin and Atherosclerotic Parameters in Chronic Hemodialysis Patients

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Key Words
Visfatin · Pulse wave velocity · Asymmetric dimethylarginine · High-sensitive C-reactive protein · Hemodialysis

Abstract
Pre-B cell colony-enhancing factor (PBEF)/visfatin is produced by adipose tissue, skeletal muscle, bone marrow, the liver and lymphocytes. Although serum PBEF/visfatin is related to the pathogenesis of atherosclerosis, and its level is elevated in patients with chronic kidney disease, it remains unclear whether increased PBEF/visfatin is associated with atherosclerotic parameters in hemodialysis (HD) patients. In this study, we measured serum PBEF/visfatin in 68 chronic HD patients (age 66 ± 14 years, time on HD 76 ± 76 months, 41 males, 27 females) and examined the association of serum PBEF/visfatin with serum asymmetric dimethylarginine, arteriosclerotic parameters such as pulse wave velocity, ankle brachial pressure index and the percent of abdominal aortic wall calcification in a cross-sectional fashion. Serum PBEF/visfatin was significantly correlated with time on HD ($r = 0.29$, $p = 0.02$), but not with age, gender and diabetes. There was no association between PBEF/visfatin and body mass index, abdominal visceral and subcutaneous fat mass area, and total adiponectin. Serum PBEF/visfatin was significantly positively correlated with log-transformed highly sensitive C-reactive protein ($r = 0.26$, $p < 0.05$) but negatively with serum albumin ($r = -0.33$, $p < 0.01$). In contrast, there was no association between serum PBEF/visfatin and asymmetric dimethylarginine, aortic pulse wave velocity, brachial ankle pressure index and percent of abdominal aortic wall calcification. It follows from these findings that serum PBEF/visfatin may reflect the inflammatory status rather than atherosclerotic changes in chronic HD patients.

Introduction

Pre-B cell colony-enhancing factor 1 (PBEF), which has recently been identified as visfatin (MW 52 kDa), is expressed in fat, skeletal muscle, the liver, bone marrow and lymphocytes [1]. PBEF/visfatin was earlier described as a growth factor enhancing the effect of interleukin (IL)-7 and stem cell factor on early stage B cells [1], but now is known to exert 3 distinct activities to cellular energetics and innate immunity. First, within the cell, PBEF/visfatin functions as a nicotinamide phosphoribosyl transferase, the rate-limiting step in a salvage pathway of nicotinamide adenine dinucleotide biosynthesis. PBEF/visfatin regulates nicotinamide adenine dinucleotide-positive-dependent reactions and promotes maturation of vascular smooth muscle cells [2]. Second, as an

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extracellular cytokine, PBEF/visfatin induces cellular expression of inflammatory cytokines such as tumor necrosis factor-α, IL-1β and IL-6 [3]. Finally, PBEF/visfatin may afford insulin-mimetic actions, although whether PBEF/visfatin binds to insulin receptor remains controversial [4].

Recently, PBEF/visfatin has been shown to directly increase endothelial adhesion molecules, such as intercellular adhesion molecule 1 and vascular cell adhesion molecule 1, through nuclear factor-κB activation in cultured human vascular endothelial cells [5]. PBEF/visfatin also activates monocyte matrix metalloproteinase 2/9, a marker of vascular inflammation [6]. In addition, PBEF/visfatin expression is upregulated in the foam cell macrophages within human unstable carotid and coronary atherosclerotic lesions [7]. PBEF/visfatin is also inversely correlated with flow-mediated vasodilatation of the brachial artery in type 2 diabetic patients [8]. Thus, PBEF/visfatin may play a causative role in atherogenesis and plaque destabilization. However, there was no study to examine a relationship between serum PBEF/visfatin and atherosclerotic parameters in hemodialysis (HD) patients.

The aim of the present study is to examine the association of PBEF/visfatin with atherosclerotic parameters in HD patients. We tested the relationship between the PBEF/visfatin and pulse wave velocity (PWV) and ankle brachial pressure index (ABI) in a cross-sectional fashion. We also examined the association of PBEF/visfatin with serum asymmetric dimethylarginine (ADMA), an independent determinant of endothelial dysfunction, and abdominal aortic wall calcification.

Subjects and Methods

Adult HD patients who gave their consent were recruited from the Maruyama clinic, an academic, tertiary referral center (Hamamatsu, Japan). Study inclusion criteria were patients who had been on regular HD therapy more than 3 months and outpatients with a clinically stable condition without any sign of infection. Of 75 enrolled participants, we excluded 7 participants from whom consent could not be obtained.

All patients had been subjected to regular HD for 4–4.5 h 3 times per week at a blood flow rate of 180–220 ml/min. All patients used bicarbonate dialysate (30 mEq/l; Kindaly AF-2P, Fuso, Osaka, Japan) at a dialysate flow rate of 500 ml/min. All HD treatments were performed using one of the following membranes: high-flux polysulfone synthetic hollow-fiber (BS-U, Toray Medical, Tokyo, Japan; APS, Asahi Medical, Tokyo, Japan, or FPX, Fresenius Medical Care, Tokyo, Japan), cellulose triacetate hollow fiber (FB-U, Nipro Medical, Tokyo, Japan) and ethylene-vinyl alcohol hollow fiber (Kf-m, Kuraray Medical, Tokyo, Japan). None of the patients reused the dialyzer. No patients were taking thiazolidinedione and statin medications which are known to reduce serum PBEF/visfatin [8, 9]. Blood samples were drawn from the arterial site of the arteriovenous fistula at the start of each dialysis session after the 2-day interval.

Analytical Procedures

Serum urea nitrogen, creatinine, calcium, phosphorus, total protein, albumin, total cholesterol, triglyceride and hemoglobin were measured by standard laboratory techniques using an autoanalyzer. Prealbumin was measured by turbidimetric immunoassay. The body mass index (BMI) was calculated by dividing dry weight (kg) by body height (m^2). Highly sensitive C-reactive protein (CRP) was quantified by means of laser nephelometry. Intact parathyroid hormone was measured by immunoradiometric assay. Serum PBEF/visfatin, total adiponectin (ADPN) and IL-6 were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (visfatin: visfatin C-terminal (human) ELISA kit, Phoenix Parmaceuticals Inc., Belmont, Calif., USA; IL-6: human IL-6 ultrasensitive ELISA, Biosource International, Camarillo, Calif., USA; total ADPN: Otsuka Pharmaceuticals, Tokyo, Japan). ADMA was also determined using ADMA direct ELISA kit (Immundiagnostik AG, Bensheim, Germany).

CT Scans

Axial CT images of the abdomen were obtained at the level of the third lumbar spine. The thickness of a slice was 10 mm. The radiographic images were digitally scanned for analysis by a personal computer. The abdominal subcutaneous fat mass area and the abdominal visceral fat mass area were measured using the public domain planimetry program, The National Institutes of Health IMAGE (written by Wayne Rasband, The National Institutes of Health, Bethesda, Md., USA) [10]. We also assessed the percentage of the aortic calcification area by measuring the calcified area of scanned abdominal aorta [10].

Assessment of Arterial Stiffness

PWV and ABI were measured in the spine position at rest before the start of the midweek dialysis session. To avoid the influence of fluid overload, we measured these parameters when the weight gain relative to body weight after last HD was below 3% of dry weight. We monitored electrocardiogram and heart sounds during the measurement. PWV from the heart to the ankle was obtained by measuring the length from the aortic valve to the ankle. To detect the brachial and ankle pulse waves with cuffs, the pressure of the cuffs was kept low at 30–50 mm Hg to ensure a minimal effect of cuff pressure on the hemodynamics. Thereafter, we measured the blood pressure at the brachial artery. All measurements and calculations of PWV and ABI were made together and automatically in CAVI-VaSera VS-1000 (Fukuda Denshi Co., Ltd., Tokyo, Japan) [10]. We repeatedly measured these parameters at both legs in each patient, and expressed them as the means.

Statistical Analysis

Values were expressed as the means ± SD. Differences between 2 groups were analyzed by an unpaired Student t test following the analysis of variance. p values <0.05 were considered statistically significant. We also examined laboratory determi-

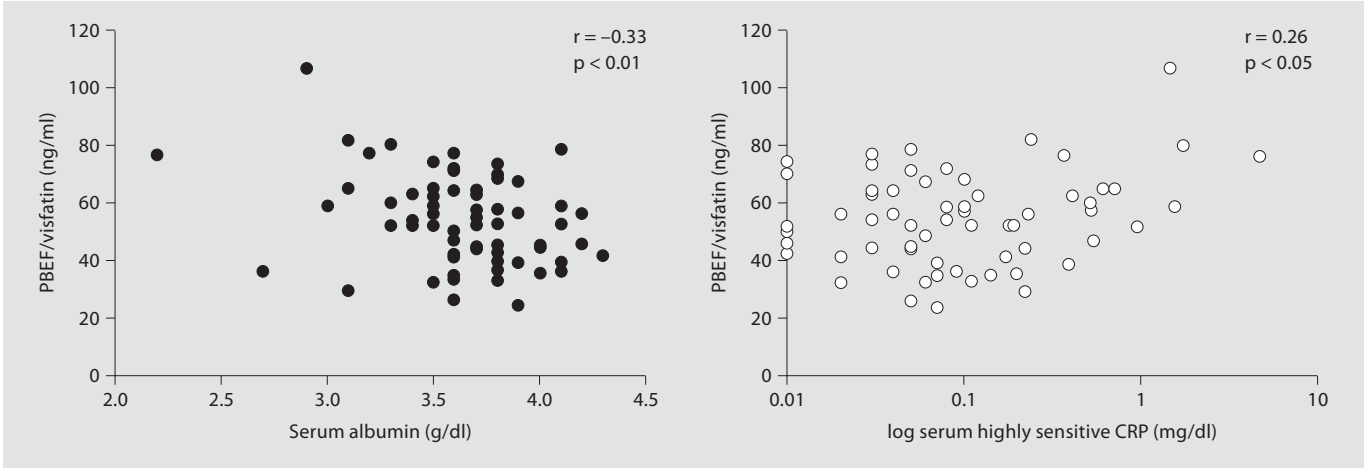

Fig. 1. Relationship between PBEF/visfatin and serum high-sensitive CRP and albumin. Serum PBEF/visfatin is significantly positively correlated with log-transformed serum highly sensitive CRP, but negatively with albumin.

Table 1. Clinical parameters

Parameters	Mean ± SD	Range
Dry weight, kg	51.5 ± 11.4	34.2–97.0
BMI	21.3 ± 3.2	15.1–29.9
Blood urea nitrogen, mg/dl	69.0 ± 13.1	28.5–101.8
Creatinine, mg/dl	10.86 ± 3.50	3.66–18.34
Calcium, mg/dl	8.9 ± 1.0	6.1–10.8
Phosphorus, mg/dl	5.8 ± 1.2	3.2–8.5
Albumin, g/dl	3.6 ± 0.4	2.2–4.3
Prealbumin, mg/dl	31 ± 8	8–46
Total cholesterol, mg/dl	154 ± 29	93–231
Triglyceride, mg/dl	122 ± 92	39–715
Intact PTH, pg/ml	197 ± 110	14–540
Hemoglobin, g/dl	10.8 ± 1.2	8.5–13.4
ASFA, cm^2	84.3 ± 44.9	8.5–228.6
AVFA, cm^2	82.1 ± 59.8	8.9–272.2
Highly sensitive CRP, mg/dl	0.27 ± 0.64	0.00–4.64
IL-6, pg/ml	2.34 ± 2.56	0.11–16.05
Total ADPN, μg/l	18.0 ± 10.0	3.5–47.7

PTH = Parathyroid hormone; ASFA = abdominal subcutaneous fat mass area; AVFA = abdominal visceral fat mass area.

Table 2. Arteriosclerotic parameters

Parameters	Mean ± SD	Range
Systolic blood pressure, mm Hg	143 ± 23	90–195
Diastolic blood pressure, mm Hg	83 ± 13	53–119
Mean arterial pressure, mm Hg	108 ± 18	70–149
PWV, m/s	18.5 ± 4.5	9.6–30.2
ABI	1.04 ± 0.17	0.51–1.26
%ACA	25.8 ± 25.1	0.0–81.0
ADMA, μM/l	0.25 ± 0.33	0.01–2.06

CAVI = Cardio-ankle vascular index; %ACA = percentage of the aortic calcification area at the abdominal aorta.

nants of PWV and ABI with multiple stepwise regression analysis. We analyzed 10 parameters: age, time on HD, serum calcium, phosphorus, albumin, intact parathyroid hormone, visfatin, total ADPN, log-transformed CRP and log-transformed IL-6. Since high-sensitive CRP and IL-6 were highly skewed, we naturally log-transformed those before the analysis. All statistical calculations were performed with Statview 5J software (SAS Institute, Cary, N.C., USA).

Results

Clinical profiles of the patients are shown in table 1. Serum PBEF/visfatin was significantly and positively correlated with HD duration ($r = 0.30$, $p = 0.01$), but did not correlate with age, gender and diabetes. Serum PBEF/visfatin was significantly and positively correlated with log-transformed highly sensitive CRP ($r = 0.26$, $p < 0.05$) and negatively with serum albumin ($r = -0.33$, $p < 0.01$; fig. 1). In contrast, there was no relationship between PBEF/visfatin and BMI, the abdominal subcutaneous fat mass area and the abdominal visceral fat mass area. Visfatin did not associate with total ADPN, total cholesterol, triglyceride and prealbumin.

Atherosclerotic parameters are shown in table 2. PWV was significantly and positively correlated with age (r =

0.55, $p < 0.01$) and log-transformed IL-6 ($r = 0.35$, $p < 0.01$). ABI was also related to age ($r = -0.42$, $p < 0.01$), albumin ($r = 0.27$, $p < 0.04$) and log-transformed IL-6 ($r = -0.28$, $p < 0.03$). However, serum PBEF/visfatin did not correlate with PWV ($r = -0.01$, $p = 0.92$) and ABI ($r = -0.03$, $p = 0.81$). There was no relationship between PBEF/visfatin and ADMA ($r = 0.08$, $p = 0.53$) and the percent of abdominal aortic wall calcification ($r = 0.18$, $p = 0.18$). Multiple stepwise regression analysis revealed that age was the only significant determinant of aortic PWV among the 10 parameters (F-to-remove 31.3, $R^2 = 0.37$). A significant indicator for ABI was albumin (F-to-remove 8.5, $R^2 = 0.14$) in this study.

Discussion

Accumulating data suggest that serum PBEF/visfatin is increased in a variety of patients with acute and chronic inflammatory status. PBEF/visfatin is exclusively expressed in neutrophils from critically ill patients with sepsis [11]. PBEF/visfatin transcription is increased in lung tissue from critically ill patients with acute lung injury [12], and serum PBEF/visfatin is also elevated in chronic inflammatory disease, including rheumatoid arthritis, inflammatory bowel disease and obesity [3, 13]. Additionally, PBEF/visfatin is positively correlated with serum IL-6 in apparently healthy subjects [14]. In this study, we observed that serum PBEF/visfatin was significantly positively correlated with highly sensitive CRP but negatively with serum albumin, confirming a close association of PBEF/visfatin with inflammation and hypoalbuminemia, as described previously in incident HD patients [15].

In contrast, PBEF/visfatin did not associate with BMI, total ADPN and abdominal fat accumulation in this study. This lack of association of PBEF/visfatin levels with abdominal adiposity, as well as with insulin resistance, has already been observed in chronic kidney disease (CKD) patients [15] and general subjects [16, 17]. Thus, these findings collectively suggest that serum PBEF/visfatin is not an adipose tissue-specific protein.

Axelsson and coworkers [15] first showed that serum PBEF/visfatin is more elevated in patients with CKD stage 5 than in those with CKD stage 3–4. They further found that serum PBEF/visfatin is positively correlated with soluble vascular cell adhesion molecule 1, a marker of endothelial damage, in patients with CKD stage 5. Recently, PBEF/visfatin has been reported to be inversely correlated with functional changes in flow-mediated vasodilatation of the brachial artery in CKD and early diabetic nephropathy [18, 19]. In this study, we measured serum ADMA, because ADMA is a significant determinant of flow-mediated vasodilatation in CKD patients [20]. Serum ADMA was significantly correlated with time on HD ($r = 0.45$, $p < 0.01$). However, no association was found between serum PBEF/visfatin and ADMA. In addition, serum PBEF/visfatin did not correlate with PWV, ABI and the percent of abdominal aortic wall calcification. These findings convincingly suggest that serum PBEF/visfatin is unrelated to atherogenic properties, including endothelial dysfunction, large artery stiffness and vascular media calcification in HD patients.

There are several limitations to this study. First, we examined the associations of serum PBEF/visfatin with atherogenic parameters in a cross-sectional fashion, and thus, our observation may mask possible cause-effect relations. In addition, this study was a single-center study analyzing a small number of patients, which affects the generalization as well as the power of the study. Second, we did not assess epicardial fat mass by echocardiography, a true visceral adipose tissue deposited around the heart. Recently, PBEF/visfatin is shown to be positively correlated with cardiac adiposity [21]. Third, we did not evaluate changes of dietary intake and body weight, which may affect PBEF/visfatin levels [22, 23]. Finally, whether increased PBEF/visfatin levels are related to adverse outcomes remains to be clarified by its cross-sectional design. However, PBEF/visfatin was not associated with the 5-year mortality after adjustment for age, gender, serum albumin, IL-6 level and glomerular filtration rate in CKD patients [15].

In summary, we found no association of serum PBEF/visfatin with serum ADMA, arterial stiffness and aortic wall calcification in HD patients. PBEF/visfatin is significantly positively correlated with log-transformed highly sensitive CRP but inversely with serum albumin. Taken together, our observations suggest that serum PBEF/visfatin may reflect the inflammatory status rather than atherosclerotic changes in chronic HD patients.

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