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Serum level of pigment epithelium-derived factor is a marker of atherosclerosis in humans

Nobuhiro Tahara[a,*], Sho-ichi Yamagishi[b,**], Atsuko Tahara[a], Yoshikazu Nitta[a], Norihiro Kodama[a], Minori Mizoguchi[a], Dilbahar Mohar[d], Masatoshi Ishibashi[c], Naofumi Hayabuchi[c], Tsutomu Imaizumi[a]

[a] Department of Medicine, Division of Cardio-Vascular Medicine, Kurume University School of Medicine, Kurume, Japan
[b] Department of Pathophysiology and Therapeutics of Diabetic Vascular Complications, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan
[c] Division of Nuclear Medicine, PET Center and Department of Radiology, Kurume University School of Medicine, Kurume, Japan
[d] Division of cardiology, University of California, Irvine, CA, USA

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ABSTRACT

Objective: Pigment epithelium-derived factor (PEDF) could play a protective role against atherosclerosis. However, there is no clinical study to examine the relationship between serum level of PEDF and atherosclerosis in humans.
Methods/results: The study involved 317 consecutive outpatients in Kurume University Hospital (220 male and 97 female) with a mean age of 62.1 ± 9.1. We examined whether serum level of PEDF were independently associated with vascular inflammation evaluated by [^{18}F]-fluorodeoxyglucose positron emission tomography (FDG-PET) and intima-media thickness (IMT) in carotid artery in humans. Carotid [^{18}F]-FDG uptake, an index of vascular inflammation within the atherosclerotic plaques, was measured as standardized uptake value (SUV). Mean serum PEDF level, carotid SUV and IMT values were 13.5 ± 1.1 μg/mL, 1.34 ± 0.19, and 0.71 ± 0.15 mm, respectively. In multiple stepwise regression analysis, estimated glomerular filtration rate ($p < 0.001$), males ($p < 0.001$), homeostasis model assessment of insulin resistance index ($p < 0.05$), heart rate ($p < 0.05$), triglycerides ($p < 0.05$), carotid IMT ($p < 0.05$), waist circumference ($p < 0.05$) and carotid SUV ($p < 0.05$) were independently correlated to PEDF level ($R^2 = 0.332$).
Conclusion: The present study reveals that serum level of PEDF is independently associated with vascular inflammation and IMT, thus suggesting that PEDF level is a novel biomarker that could reflect atherosclerosis in humans.

1. Introduction

Pigment epithelium-derived factor (PEDF), a glycoprotein that belongs to the superfamily of serine protease inhibitors, was first purified from the conditioned media of human retinal pigment epithelial cells as a factor which possesses potent neuronal differentiating activity [1]. PEDF has been shown to be a highly effective inhibitor of angiogenesis in cell culture and animal models; PEDF inhibited retinal endothelial cell (EC) growth and migration and suppressed ischemia-induced retinal neovascularization [2,3]. In addition, PEDF levels in aqueous humor or vitreous are decreased in diabetic patients, especially with proliferative retinopathy, thus suggesting that loss of PEDF in the eye is functionally important in the pathogenesis of angiogenic eye diseases [4].

Recently, we have found that PEDF inhibits cytokine-, growth factor- and advanced glycation end product-induced EC damage, smooth muscle cell proliferation and migration, macrophage and T cell activation, and platelet hyperaggregation *in vitro* through its anti-oxidative and anti-inflammatory properties [5–10]. In addition, PEDF has been reported to reduce vascular inflammation and hyperpermeability in experimental diabetic retinas, suppress carotid artery thrombus formation in a rat model of arterial occlusion, and prevent vascular remodeling after balloon angioplasty in rats [7–10]. These observations suggest that PEDF may play a

Abbreviations: PEDF, pigment epithelium-derived factor; EC, endothelial cell; FDG-PET, [^{18}F]-fluorodeoxyglucose-positron emission tomography; IMT, intima-media thickness; BP, blood pressure; LDL-cholesterol, low-density lipoprotein-cholesterol; HDL-cholesterol, high-density lipoprotein-cholesterol; HbA1c, glycosylated hemoglobin; hs-CRP, high-sensitivity C-reactive protein; ELISA, enzyme-linked immunosorbent assay; HOMA-IR, homeostasis model assessment of insulin resistance; GFR, glomerular filtration rate; CT, computed tomography; SUV, standardized uptake value; SD, standard deviation.

* Corresponding author at: 67 Asahi-machi, Kurume, 830-0011, Japan. Tel.: +81 942 317580; fax: +81 942 317707.
** Corresponding author at: 67 Asahi-machi, Kurume, 830-0011. Tel.: +81 942 317873; fax: +81 942 317895.
E-mail addresses: ntahara@med.kurume-u.ac.jp (N. Tahara), shoichi@med.kurume-u.ac.jp (S.-i. Yamagishi).

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protective role against vascular inflammation and atherosclerosis *in vivo*.

PEDF is produced from a variety of tissues, including vascular cells, inflammatory cells and adipocytes [10–12]. We have previously shown that serum PEDF level is elevated in proportion to the accumulation of the number of the components of the metabolic syndrome in a general population [13]. However, the relationship between serum level of PEDF and atherosclerosis in humans remains unknown. Therefore, in this study, we examined whether serum level of PEDF is independently correlated with vascular inflammation evaluated by [^{18}F]-fluorodeoxyglucose positron emission tomography (FDG-PET) and intima-media thickness (IMT) in carotid artery in humans.

2. Methods

2.1. Subjects

Three hundred and seventeen consecutive outpatients (220 males and 97 females, 62.1 ± 9.1 years old) who visited at Kurume University Hospital for a medical screening of atherosclerosis were enrolled in this study. The number of patients who received aspirin, statins, anti-hypertension drugs and medications for diabetes were 57, 38, 128 and 24, respectively. Thirty-two patients had angiographically documented coronary artery disease and/or a history of coronary vascular events. Seventeen subjects had a radiographically documented cerebrovascular disease. We excluded any patients with inflammatory, neoplastic disorders, and any acute infection. All participants gave informed consent to participate in this study. The Ethical Committee for the Clinical Research of Kurume University approved this study.

2.2. Data collection

The medical history and use of smoking were ascertained by a questionnaire. Smoking was classified as current habitual use or not. Waist circumference was measured as an index of the presence or absence of central obesity. Blood pressure (BP) was measured in the sitting position using an upright standard sphygmomanometer. Vigorous physical activity and smoking were avoided for at least 30 min before BP and resting heart rate measurements.

Blood was drawn after 12-h fasting from the antecubital vein in the morning for determinations of lipid profiles [total-cholesterol, low-density lipoprotein-cholesterol (LDL-cholesterol), triglycerides, and high-density liporprotein-cholesterol (HDL-cholesterol)], plasma glucose, insulin, glycosylated hemoglobin (HbA1c), blood urea nitrogen, creatinine, uric acid, high-sensitive C-reactive protein (hs-CRP). These blood chemistries were measured with standard methods at a commercially available laboratory (The Kyodo Igaku Laboratory, Fukuoka, Japan) as described previously [13]. Serum PEDF measurements were performed with the competitive enzyme-linked immunosorbent assay (ELISA) as described previously [14]. In brief, a 96-well microtiter plate was coated by overnight incubation at 4 °C with 5 μg/mL anti-PEDF monoclonal antibody (Transgenic, Kumamoto, Japan). Fifty microliters of serum was pretreated with 200 μL of 8 M urea at 4 °C for 1 h and then each sample was 50-fold diluted with 10 mM phosphate-buffered saline. After washing the microtiter plate, 100-μL aliquots of standard recombinant human PEDF proteins (0.5–300 ng/mL; Chemicon International, Temecula, CA, USA) or diluted serum were added to the wells and then the plate was incubated at room temperature for 2 h. Then the well was washed four times, and 50 μL of biotinylated anti-human PEDF polyclonal antibody (R&D Systems, Minneapolis, MN, USA) was added to each well. After 2-h incubation at room temperature, the plate was incubated with 100 μL of HRP-conjugated streptavidin solution (Zymed, South San Francisco, CA, USA) at room temperature for 30 min. Then the well was washed again, and 50 μL of chromogenic substrate solution (Dako, Tokyo, Japan) was added to each well and the plate was incubated with shaking at room temperature for 15 min. After the color was developed, 50 μL of reaction stopper was added. The plate was read at 450 nm using a microplate reader (Emax, Molecular Devices, Bucher Biotec AG-Basel, Switzerland). Inter- (n = 17) and intra-assay (n = 14) coefficient of variations of the ELISA were 4.7% and 7.3%, respectively.

Insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR). HOMA-IR index was calculated from the values of fasting plasma glucose (mg/dL) and fasting insulin (μU/mL) using the following formula [(glucose × insulin)/405]. Estimation of glomerular filtration rate (GFR) was calculated using the with the Cockcroft-Gault equation [15].

2.3. Measurement of carotid artery IMT

Carotid artery IMT was determined as a parameter of atherosclerosis. IMT of the common carotid artery was determined using duplex ultrasonography with a 10-MHz transducer (SSA-380A, Toshiba, Tokyo, Japan) according to the method described previously [16,17]. In brief, longitudinal B-mode images at the diastolic phase of cardiac cycles were recorded by a single trained technician who was blinded to the subjects' background. Measurements of carotid IMT were made by the same technician using fine slide calipers at 3 levels of the lateral and medial walls 1–3 cm proximal to the carotid bifurcation. The mean of these 6 measurements was taken as the value for the carotid IMT. The intra-observer or inter-observer variability of IMT measurements was less than 5%.

2.4. FDG-PET imaging

FDG-PET imaging was performed as described previously [16,17]. In brief, after at least 12 h-fasting, the study patients received an intravenous administration of FDG (4.2 MBq (0.12 mCi)/kg body weight). One hour after the FDG injection, 3-dimensional whole-body PET imaging was carried out using a PET scanner (Allegro, Philips Medical Systems [Cleveland], Inc., Cleveland, Ohio, USA). We performed an attenuation correction for the PET imaging by a rotating rod of activity in the PET scanner. Contrast enhanced computed tomography (CT) images were also taken from the skull base to the diaphragm using Light Speed Ultra 16 (GE Healthcare, Milwaukee, Wisconsin). To overcome spatial resolution limitations of PET in this study, co-registration of PET and CT imaging (PET/CT imaging) was performed for review on a workstation (Sun Microsystems, Inc., Santa Clara, California). The intensity of FDG uptake was quantified by measuring the standardized uptake value (SUV) corrected for body weight. The SUV was calculated by using the maximum pixel activity value within the region of interest placed on the vascular wall of the transaxial PET/CT image. The arterial SUV score was determined as the average of the SUVs of both the common carotid arteries obtained from 10 consecutive PET/CT images, each separated by 4 mm in length with the most cranial site starting at the carotid bifurcation. Two blinded radiologists measured the SUV values. The intra-observer or inter-observer variability of SUV measurements was less than 5%.

2.5. Statistical analysis

Data were described as mean ± standard deviation (SD). Discontinuous variables were coded as dummy variables. Because of skewed distributions, the natural logarithmic transformations were performed for triglycerides, fasting plasma glucose, insulin,