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Serum levels of pigment epithelium-derived factor (PEDF) are independently associated with procollagen III N-terminal peptide levels in patients with nonalcoholic fatty liver disease

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ABSTRACT

Objectives: Pigment epithelium-derived factor (PEDF) is a glycoprotein that belongs to the superfamily of serine protease inhibitors with complex anti-oxidative, anti-fibrotic, and anti-inflammatory properties, thus being involved in cardiometabolic disorders. Nonalcoholic fatty liver disease (NAFLD) is a hepatic manifestation of the metabolic syndrome as well. However, the pathophysiological role of PEDF in NAFLD remains largely unknown. We studied here the relationship between serum PEDF levels and various clinical markers of NAFLD in humans.

Design and methods: The study involved 194 biopsy-proven NAFLD patients (102 male and 92 female) with a mean age of 51.3 ± 13.8 years. We examined which anthropometric, metabolic and inflammatory variables, and liver steatosis and fibrosis markers are independently associated with serum levels of PEDF.

Results: Mean serum levels of PEDF were 16.4 ± 5.7 μg/mL. Univariate analysis revealed that age (inversely), male, body mass index, waist circumference, numbers of white blood cells and platelets, aspartate aminotransferase, alanine aminotransferase, fasting plasma glucose, glycated hemoglobin, uric acid, procollagen type III N-terminal peptide (P-III-P), subcutaneous fat areas, visceral fat areas and liver to spleen density ratio in computed tomography, the presence of diabetes and medication for hyperlipidemia were significantly associated with serum levels of PEDF. In multiple stepwise regression analysis, age ($p<0.01$, inversely), male ($p<0.05$), waist circumference ($p<0.01$), white blood cell number ($p<0.05$), P-III-P ($p<0.05$), and the presence of diabetes ($p<0.05$) and medication for hyperlipidemia ($p<0.01$), were independently correlated to serum levels of PEDF ($R^2 = 0.285$).

Conclusions: The present study reveals that serum levels of PEDF are independently associated with P-III-P levels, suggesting that PEDF level is a novel biomarker of liver fibrosis in patients with NAFLD.

Introduction

Nonalcoholic fatty liver disease (NAFLD) encompasses a broad spectrum of conditions, ranging from simple steatosis to nonalcoholic steatohepatitis [1–3]. The latter is recognized as a potentially progressive disease that could lead to cirrhosis, liver failure, and hepatocellular carcinoma [1–3]. NAFLD has been considered to be a hepatic manifestation of the metabolic syndrome and particularly correlated with insulin resistance, visceral obesity, hypertension, and abnormalities in glucose and lipid metabolism [4,5]. These underlying metabolic derangements account for the increased risk for liver fibrosis and advanced liver disease in patients with cardiovascular disorders [6]. The 'two-hit theory' best describes the progression from simple steatosis to nonalcoholic steatohepatitis, which is consisted of the 'first' accumulation of excessive fat in the liver due to increased visceral adipocyte lipolysis and 'second hit' triggered by oxidative stress and inflammation [4,7–9].

Pigment epithelium-derived factor (PEDF) is a glycoprotein that belongs to the superfamily of serine protease inhibitors with a potent neuronal differentiating activity [10]. PEDF is found to be a highly effective inhibitor of pathological angiogenesis in both cell culture and animal models [11,12]. Furthermore, it has also been shown to have neuroprotective, anti-oxidative and anti-inflammatory properties, any

Abbreviations: NAFLD, nonalcoholic fatty liver disease; PEDF, pigment epithelium-derived factor; HT, hypertension; DM, diabetes mellitus; HL, hyperlipidemia; T-Chol, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein-cholesterol; Hb, hemoglobin; WBC, white blood cells; FPG, fasting plasma glucose; IRI, fasting plasma insulin; HbA1c, glycated hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; FFA, free fatty acid; P-III-P, procollagen III N-terminal peptide; hsCRP, high-sensitivity C-reactive protein; ELISA, enzyme-linked immunosorbent assay; HOMA-IR, homeostasis model assessment of insulin resistance; CT, computed tomography; VFA, visceral fat areas; SFA, subcutaneous fat areas; L/S, liver to spleen; NAS, NAFLD activity score; SD, standard deviation.

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of which could potentially be exploited as a therapeutic option for the treatment of various cardiometabolic disorders, neurodegenerative disease and cancers [13–18]. Indeed, we, along with others, have recently found that PEDF not only inhibits advanced glycation end product-induced hepatic insulin resistance *in vitro*, but also suppresses hepatic steatosis in PEDF null mice [19–21]. However, the relationship between serum PEDF levels and NAFLD remains unclear. In this study, we examined which anthropometric, metabolic and inflammatory variables, and liver steatosis and fibrosis markers are independently associated with serum levels of PEDF in patients with NAFLD.

Methods

Subjects

A total of 194 consecutive biopsy-proven NAFLD patients (102 males and 92 females, 51.3 ± 13.8 years old) were enrolled in the present study. Patients were introduced to our hospital for closer examination of liver biological abnormalities. All patients were negative for serology and viral hepatitis, and had no history of liver diseases. Current and past daily alcohol intake of the subjects was less than 20 g per week. Furthermore, we excluded any patients with drug-induced hepatitis, autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, Wilson's disease and biliary obstruction. The number of patients who received anti-hypertension (HT) drugs and medications for diabetes mellitus (DM) and hyperlipidemia (HL) were 75, 58 and 77 respectively. Ninety-six patients had type 2 DM. All participants gave informed consent to participate in this study. The Ethical Committee for Clinical Research of Hiroshima University approved this study. The study complied with the principles of Ethical Publishing in the Helsinki Declaration [22].

Data collection

Blood was drawn after 12-hour fasting from the antecubital vein in the morning for determinations of lipid profiles; total cholesterol (T-Chol), triglycerides (TG), and high-density lipoprotein-cholesterol (HDL-C), hemoglobin (Hb), white blood cells (WBC), platelets, fasting plasma glucose (FPG), fasting plasma insulin (IRI), glycated hemoglobin (HbA1c), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), uric acid, total protein, albumin, total-bilirubin, direct-bilirubin, free fatty acid (FFA), type IV collagen, hyaluronic acid, procollagen III N-terminal peptide (P-III-P), ferritin and high-sensitivity C-reactive protein (hsCRP). These blood chemistries were measured with standard enzymatic methods or enzyme-linked immunosorbent assay (ELISA) kits as described previously [23,24]. Serum PEDF measurements were performed with the competitive ELISA as described previously [25,26]. 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 FPG (mg/dL) and IRI (μU/mL) using the following formula [(FPG × IRI)/405]. A 75-g OGTT was performed, and plasma glucose and insulin levels were analyzed periodically for 2 h after glucose loading.

Within a couple of weeks after the 75-g OGTT, computed tomography (CT) scanning was performed for quantitatively measuring visceral and subcutaneous fat areas (VFA and SFA) at the level of the umbilics and fat content in the liver. VFA and SFA were identified as described previously [27]. Liver fat content was shown as CT density ratio of liver to spleen (L/S density ratio) as described previously [28]. Then, all patients underwent a percutaneous liver biopsy under ultrasonic guidance. The specimens were subsequently fixed in formalin and embedded in paraffin. All the specimens were examined by an experienced pathologist, who was unaware of the clinical and biochemical data of the patients. All cases showed macrovesicular steatosis affecting at least 5% of hepatocytes and were classified as either steatosis or steatohepatitis. In addition to steatosis, minimum criteria for the diagnosis of steatohepatitis included the presence of lobular inflammation and either ballooning cells or perisinusoidal/pericellular fibrosis in zone 3 of the hepatic acinus as described previously [27]. NAFLD activity score (NAS) was calculated as the unweighted sum of the scores for steatosis, lobular inflammation, and ballooning as reported by Kleiner et al. [29]. Steatosis grade and fibrosis stage were evaluated as described previously [27]. In brief, steatosis was graded as follows: grade 1 (≥5% and <33% of hepatocytes affected), grade 2 (33–66% of hepatocytes affected), or grade 3 (<66% of hepatocytes affected). Fibrosis was graded 0 (absent) to 4 (1, perisinusoidal/pericellular fibrosis; 2, periportal fibrosis; 3, bridging fibrosis; and 4, cirrhosis).

Statistical analysis

Data are described as mean ± standard deviation (SD). A correlation between PEDF and various clinical variables was determined by a linear regression analysis. To determine the independent parameters related to serum PEDF levels, multiple stepwise regression analysis was performed. Statistical significance was defined as $p<0.05$. All statistical analyses were performed using the SPSS system (SPSS Inc., Chicago, IL, USA).

Results

Demographical data of the subjects are presented in Table 1. Mean serum levels of PEDF were 16.4 ± 5.7 μg/mL. Univariate analysis revealed that age (inversely), male, body mass index, waist circumference, numbers of WBC and platelets, AST, ALT, FPG, HbA1c, uric acid, P-III-P, SFA, VFA and L/S density ratio in CT, the presence of DM and medication for HL were significantly associated with serum levels of PEDF (Table 2). Because these significant parameters could be closely correlated with each other, we performed multiple stepwise regression analysis in order to determine the independent correlates of PEDF levels. As shown in Table 2, age ($p<0.01$, inversely), male ($p<0.05$), waist circumference ($p<0.01$), WBC ($p<0.05$), P-III-P ($p<0.05$), and the presence of DM ($p<0.05$) and medication for HL ($p<0.01$) remained significant and were independently correlated to PEDF levels ($R^2 = 0.285$).

Discussion

As far as we know, there exists only one published paper of Yilmaz et al. [30], which investigated the relationship between serum levels of PEDF and NAFLD. They showed that compared with control subjects, serum PEDF levels were increased in patients with NAFLD and independently associated with liver steatosis [30]. Therefore, in this study, we comprehensively examined which anthropometric, metabolic and inflammatory variables as well as liver steatosis and fibrosis markers were independently correlated with serum PEDF levels in biopsy-proven NAFLD patients. We found here for the first time that P-III-P, a marker of liver fibrosis [23,24], was independent correlates of serum PEDF levels in our patients. We, along with others, have previously shown that administration of PEDF not only inhibits fibrotic reactions in the kidney of diabetic rats [31,32], but also prevents cardiac fibrosis and remodeling in rats with acute myocardial infarction [33], both of which were partly mediated by suppression of transforming growth factor-β expression. Further, delivery of PEDF gene into the liver has been reported to ameliorate hepatic fibrosis in mice treated with carbon tetrachloride or thioacetamide [34]. Moreover, compared with wild-type animals, expression levels of fibrotic markers, including transforming growth factor-β, were higher in PEDF-null mice at baseline, and experimental pancreatits-induced fibrotic responses were more enhanced in these mice [35]. These findings suggest that serum PEDF levels may be elevated as a counter-system against fibrotic reactions and could be a novel biomarker of liver fibrosis in patients with NAFLD.