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Low serum level of secreted frizzled-related protein 5, an anti-inflammatory adipokine, is associated with coronary artery disease

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ABSTRACT

Objective: Secreted frizzled-related protein 5 (SFRP5) is an anti-inflammatory adipokine that is associated with insulin resistance in animals. To extend these observations to humans, we investigated the association of serum SFRP5 levels in subjects with and without coronary artery disease (CAD).
Methods: Subjects ($n = 185$, 68 ± 11 years, 79% male) suspected of having CAD were enrolled in the study and were divided into two groups, CAD and non-CAD subjects, according to the results of their coronary angiographies. Serum SFRP5 levels of the subjects were measured by an enzyme-linked immunosorbent assay.
Results: The serum SFRP5 levels in the subjects with CAD were significantly lower than those in the non-CAD subjects (median [interquartile range]: 47.7 [26.6] vs. 52.4 [29.6] ng/mL, respectively; $p = 0.02$). The serum SFRP5 levels significantly correlated with body mass index, the homeostasis model of assessment of insulin resistance, adiponectin levels, and CAD severity. Multivariate logistic regression analysis revealed that a decreased serum SFRP5 level (log transformed) was independently associated with CAD for all subjects (adjusted odds ratio, 0.36; 95% confidence interval, 0.14–0.94; $p = 0.03$).
Conclusion: Serum SFRP5 levels are significantly associated with CAD in humans, suggesting that low SFRP5 levels may contribute to CAD.

1. Introduction

Inflammation plays a pivotal pathogenic role in obesity-related diseases, such as type 2 diabetes, metabolic syndrome, and atherosclerosis [1–3]. Adipose tissue functions as a major endocrine organ by mediating adipokine levels in a number of signaling cascades in tissues that exhibit pro-inflammatory or anti-inflammatory activity [4]. Therefore, targeting the molecular mechanisms that lead to dysregulation of adipokines may provide a novel therapeutic strategy for the treatment of inflammation-related metabolic disorders and cardiovascular disease [5].

Secreted frizzled-related protein 5 (SFRP5), a recently discovered protein secreted by adipocytes, is involved in inflammation and insulin resistance (IR) in mouse models of obesity and type 2 diabetes mellitus [6]. Sfrp5-deficient mice that are fed a high-calorie diet are severely glucose intolerant and have hepatic steatosis, which leads to inflammation of adipose tissue. One study showed that SFRP5 levels are decreased in obese humans with type 2 diabetes mellitus [7]. However, another study found no difference in circulating SFRP5 between lean and obese subjects [8]. Thus, our understanding of the effects of SFRP5 on human physiology is limited.

For the present study, we aimed to clarify the clinical significance of circulating SFRP5 levels in the context of coronary artery disease (CAD). We determined the circulating SFRP5 levels in subjects who did or did not have CAD and then evaluated the

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relationship between those levels and cardiometabolic risk factors. In addition, we also studied the relationship between serum SFRP5 levels and CAD according to the ages of the subjects.

2. Materials and methods

2.1. Subjects

The study population consisted of 185 (146 men and 39 women, mean age 68 years) consecutive patients who underwent elective coronary angiography at Kagawa Prefectural Central Hospital, Japan from November 2009 to November 2010. Coronary angiography was performed by physician's decision on the basis of angina-like chest symptoms and/or non-invasive test results consistent with myocardial ischemia. Patients with a previous history of coronary intervention or coronary artery bypass graft surgery were excluded to avoid artificial bias from such procedures. We also excluded patients with chronic hemodialysis, acute coronary syndrome, recent myocardial infarction (within 4 weeks of enrollment), inflammatory disease such as collagen disease, and malignant disease. The patients were divided into two groups on the basis of their coronary angiography: subjects with significant coronary stenosis defined as >50% narrowing of the luminal diameter (128 patients with stable CAD) and those with <50% coronary stenosis (57 patients without CAD). The Ethics Committee of Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences and Kagawa Prefectural Central Hospital approved this study. Written, informed consent was obtained from all subjects before the start of the study. The investigation conformed to the principles outlined in the Declaration of Helsinki.

2.2. Coronary angiography

Coronary angiographies were performed according to standard methods. After intracoronary injection of isosorbide dinitrate, angiograms were obtained in two or more views. Each coronary angiogram was scored by two independent investigators using two criteria [9]. The diseased vessel score was assigned as 0–3 depending on the number of coronary vessels having >50% angiographic narrowing for the left main, the left anterior descending, the left circumflex, and the right coronary arteries. Angiographic narrowing of >50% of the left main artery was scored as multi-vessel disease. In addition, a modified Gensini score was calculated [10]. Briefly, the most severe stenosis in each of eight segments was graded according to its severity, from 1 to 4. The scores for the eight segments were added to give a total score.

2.3. Definitions of coronary risk factors

Diabetes was confirmed according to the criteria of the American Diabetes Association [11] or based on a history of diabetes mellitus treatment. Dyslipidemia was defined using one or more of the following: (1) ≥150 mg/dL serum triglyceride, (2) <40 mg/dL high-density lipoprotein (HDL)-cholesterol, (3) ≥140 mg/dL low-density lipoprotein (LDL)-cholesterol, and (4) current treatment with a lipid-lowering drug. Hypertension was defined as having a seated blood pressure of ≥140/90 mmHg or undergoing current treatment with antihypertensive medication. Smoking status was defined as currently smoking or not smoking. The estimated glomerular filtration rate (eGFR) was calculated using the modification of diet in renal disease equation [12] with coefficients modified for Japanese subjects [13] as follows: eGFR (mL/min/1.73 m^2) = 194 × (serum creatinine concentration)$^{-1.094}$ × (age)$^{-0.287}$ (×0.739 for female participants). Renal dysfunction was defined as an eGFR of <60 mL/min/1.73 m^2.

2.4. Clinical and biochemical assessments

Blood samples were taken after overnight fasting. Serum was isolated and stored at −80 °C. Levels of serum SFRP5 (Human ELISA kit, Wuhan USCN Science Co, Ltd, China), adiponectin (Sekisui Medical, Japan), and hs-CRP (R&D Systems, Minneapolis, MN) were measured by enzyme-linked immunosorbent assays [14]. The coefficients of variation for the intra-assays were <10, <5, and <8%, and those for the inter-assays were <13, <5, and <7% for SFRP5, adiponectin, and hs-CRP levels, respectively.

2.5. Statistical analyses

Continuous variables are presented as the mean ± SD or median (interquartile range), and the differences between the two groups were evaluated with an unpaired *t*-test or the Mann–Whitney *U*-test, as appropriate. Categorical variables are presented as frequency counts, and intergroup comparisons were analyzed using the χ^2 test. Data that were not normally distributed, as determined by the Kolmogorov–Smirnov test, were logarithmically transformed before linear regression analysis and logistic regression analysis. Associations between the presence of CAD and clinical/biochemical parameters (including age; gender; smoking status; the presence of hypertension, diabetes mellitus, and dyslipidemia; and serum SFRP5, adiponectin, and hs-CRP levels) were analyzed by multivariate logistic regression analysis. The receiver operating characteristic curve (ROC curve) was used to obtain the specificity and sensitivity of the serum SFRP5 for distinguishing patients with CAD from non-CAD patients. Statistical significance was defined as $p < 0.05$. Statistical analysis was performed using SPSS 11.0 for Windows (SPSS Inc., Chicago, IL).

3. Results

3.1. Characteristics of study participants

Table 1 shows the baseline characteristics of the subjects. No differences were found for CAD and non-CAD subjects regarding age; gender; the prevalence of hypertension, dyslipidemia, or diabetes; smoking status, and prescribed medications. Serum SFRP5 levels in CAD subjects were significantly lower than in non-CAD subjects (Table 1; Fig. 1A). Regarding the relationship between the number of atherosclerotic lesions and serum SFRP5, serum SFRP5 levels in subjects with multiple diseased vessels ($n = 47$) were significantly lower than in subjects with a single diseased vessel ($n = 81$; 43.3 [23.6] and 51.5 [30.5] ng/mL, expressed as the median [interquartile range], respectively; $p < 0.01$). Gensini scores, which are indicators of CAD severity, were significantly correlated with log-transformed serum SFRP5 levels ($r = -0.20$, $p < 0.01$). Levels of hs-CRP were significantly higher in CAD subjects than in non-CAD subjects (Table 1). Adiponectin levels in CAD subjects were significantly lower than in non-CAD subjects (Table 1). No significant differences in serum SFRP5 levels were associated with age; gender; smoking status; and the presence of hypertension, diabetes, or dyslipidemia (Table 2). Regarding medications, significant differences in serum SFRP5 levels were associated with the use of diuretics and thiazolidinediones, but not with angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (ACEI/ARBs), calcium channel blockers (CCB), β-blockers, or statins (Table 2).

3.2. Relationships between serum SFRP5 levels and biochemical parameters

For all participants, serum SFRP5 levels were significantly and negatively correlated with body mass index (BMI), the homeostasis