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sRAGE is associated with low waist circumference and Hb levels in NAFLD

Research Article

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Abstract: Advanced glycation end products (AGEs) and the receptor RAGE interaction is involved in nonalcoholic fatty liver disease (NAFLD). Although exogenously administered soluble RAGE (sRAGE) has been shown to block the harmful effects of AGEs in animal models, there is still controversy about the role of sRAGE in humans. We examined here which anthropometric, metabolic and clinical variables were independent correlates of sRAGE levels in NAFLD patients. The study involved 77 biopsy-proven, unmedictaed NAFLD patients (44 male and 33 female) with a mean age of 43.4±13.0 years old. We examined which anthropometric, metabolic and clinical variables, including liver steatosis and fibrosis markers, are independently associated with serum levels of sRAGE. Mean serum levels of sRAGE were 710.7±290.2 pg/mL. Univariate analysis revealed that waist circumference (inversely), hemoglobin (inversely), number of white blood cells (inversely), total-bilirubin (inversely), free fatty acid (inversely), ferritin (inversely), and HbA1c (inversely) were significantly correlated with serum levels of sRAGE. In multiple stepwise regression analysis, waist circumference ($p<0.01$, inversely) and hemoglobin ($p<0.01$, inversely) were independently associated with serum levels of sRAGE ($R^2=0.176$). The present study reveals that low serum levels of sRAGE are independently associated with waist circumference and hemoglobin in patients with NAFLD.

Keywords: *sRAGE • NAFLD • Hemoglobin • Liver • Obesity*

1. Introduction

Reducing sugars can react non-enzymatically with amino groups of protein to form Amadori products [1]. These early glycation products undergo progressive modification over time *in vivo* to the formation of irreversible cross-links, after which the molecules are termed "advanced glycation end products (AGEs)" [1]. There is accumulating evidence that AGEs and their receptor RAGE interaction is involved in various cardiometabolic disorders, including nonalcoholic fatty liver disease (NAFLD) [1-4].

Recently, soluble RAGE (sRAGE) has been identified in human serum [5]. Since exogenously administered sRAGE has been shown to block the harmful effects of AGEs in animal models [5], it is conceivable that sRAGE may work as a decoy receptor. However, there is still controversy about the pathophysiological role of sRAGE in humans [5]. Some papers have shown that sRAGE levels could reflect tissue RAGE expression and predict future cardiovascular events, while the others have reported that sRAGE levels are decreased in patients with cardiovascular disease [5]. Therefore, in this study, we examined which anthropometric, metabolic and clinical variables, including liver steatosis and fibrosis markers, were independent correlates of sRAGE levels in biopsy-proven, unmedicated nonalcoholic fatty liver disease (NAFLD) patients.

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2. Patients and Methods

2.1. Subjects

A total of 77 consecutive biopsy-proven, unmedicated NAFLD patients (44 males and 33 females, 43.4±13.0 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. 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.

2.2. 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), glycated hemoglobin (HbA1c), aspartate aminotransferase (AST), alanine aminotransferase (ALT), g-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 [3]. Serum sRAGE measurements were performed with the competitive enzyme-linked immunosorbent assay as described previously [6]. Homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated using the following formula [(FPG (mg/dL) ´ fasting insulin (μU/mL))/405]. A 75-g OGTT was performed, and plasma glucose and insulin levels were analyzed periodically for 2 hours 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 [3]. Liver fat content was shown as CT density ratio of liver to spleen (L/S density ratio) as described previously [3]. Then, all patients underwent a percutaneous liver biopsy under ultrasonic guidance. NAFLD activity score (NAS) was calculated as the unweighted sum of the scores for steatosis, lobular inflammation, and ballooning as described previously [3].

2.3. Statistical analysis

Data are described as mean ± standard deviation. A correlation between sRAGE and various clinical variables was determined by a linear regression analysis. To determine the independent parameters related to serum sRAGE 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).

3. Results

Demographical data of the subjects are presented in Table 1. Mean serum levels of sRAGE were 710.7±290.2 pg/mL. Univariate analysis revealed that waist circumference (inversely), Hb (inversely), number of WBC (inversely), total-bilirubin (inversely), FFA (inversely), ferritin (inversely), and HbA1c (inversely) were significantly associated with serum levels of sRAGE (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 sRAGE levels. As shown in Table 2, waist circumference (inversely, $p<0.01$) and Hb (inversely, $p<0.01$) remained significant and were independently correlated to sRAGE levels ($R2=0.176$).

4. Discussion

As far as we know, there exists only one published paper of Yilmaz et al. [7], which investigated the relationship between serum levels of sRAGE and NAFLD in adults. They showed that compared with control subjects, serum sRAGE levels were decreased in patients with definite and borderline nonalcoholic steatohepatitis and associated with AST and ALT, which was independent of HOMA-IR. Further, they reported that that sRAGE levels below 1309 pg/mL were independently associated with the presence of nonalcoholic steatohepatitis. However, in this cohort, multiple stepwise regression analysis for the determinants of sRAGE was not performed Therefore, we comprehensively examined which anthropometric, metabolic and clinical variables,

Table 1. Clinical variables of patients

Characteristics	
Age (years)	43.4±13.0
Number (male number)	77(44)
sRAGE (pg/mL)	710.7±290.2
Body mass index (kg/m^2)	28.1±3.9
Waist circumference (cm)	94.6±8.9
Hb (g/dL)	15.0±1.5
WBC (x10^3/mL)	6014.2±1482.4
Platelets (104/mL)	23.0±4.9
AST (IU/L)	49.3±29.1
ALT (IU/L)	91.1±59.6
γ-GTP (IU/L)	88.8±84.9
Total-bilirubin (mg/dL)	0.95±0.41
Direct-bilirubin (mg/dL)	0.18±0.11
Albumin (g/dL)	4.73±0.42
T-Chol (mg/dl)	227.0±40.4
TG (mg/dl)	159.6±75.6
HDL-C (mg/dl)	51.6±14.0
Uric acid (mg/dL)	5.86±1.41
FFA (mEq/L)	0.57±0.25
Type IV collagen (ng/mL)	4.01±0.97
Hyaluronic acid (ng/mL)	43.1±118.0
P-III-P (U/mL)	0.61±0.25
Ferritin (ng/mL)	187.8±136.4
hsCRP (mg/L)	0.30±0.55
SFA (cm^2)	222.5±93.3
VFA (cm^2)	128.0±47.4
NAS	4.64±1.62
L/S density ratio	0.75±0.34
HbA1c (%)	6.13±0.86
FPG (mg/dL)	110.3±22.9
HOMA-IR	5.41±11.26
Insulinogenic index	0.96±0.87
NGT Number	34
IGT Number	23
DM Number	20

Data are shown as mean±SD, unless otherwise indicated.

sRAGE; soluble form of receptor for advanced glycation end products, Hb; hemoglobin, WBC; white blood cells, AST; aspartate aminotransferase, ALT; alanine aminotransferase, γ-GTP; γ-glutamyl transpeptidase, T-Chol; total cholesterol, TG; triglycerides, HDL-C; high-density lipoprotein cholesterol, FFA; free fatty acid, P-III-P; procollagen III N-terminal peptide, hsCRP; high-sensitivity C-reactive protein, SFA; subcutaneous fat areas, VFA; visceral fat areas, NAS; nonalcoholic fatty liver disease activity score, L/S; liver to spleen, HbA1c; glycated hemoglobin, FPG; fasting plasma glucose, HOMA-IR; homeostasis model assessment of insulin resistance, NGT; normal glucose tolerance, IGT; impaired glucose tolerance, DM; diabetes mellitus

including liver steatosis and fibrosis markers, were independently correlated with serum sRAGE levels in biopsy-proven, unmedicated NAFLD patients. In this study, although number of WBC (inversely), total-bilirubin (inversely), FFA (inversely), ferritin (inversely), and HbA1c (inversely) were significantly associated with serum levels of sRAGE in univariate analysis (Table 2), the significance was lost after multiple stepwise regression analysis. We found here for the first time that waist circumference and Hb values were independent correlates of decreased serum sRAGE levels in our patients. In the present study, compared with the control subjects (19 male and 36 female; mean age, 55.6 ± 14.9 years old, body mass index 22.5±2.8) without liver disease, diabetes or cardiovascular disease, sRAGE levels were significantly lower in patients with NAFLD (828.5±361.8 vs. 710.7±290.2 pg/mL, p<0/05). Therefore, low serum levels of sRAGE in NAFLD patients may be partly explained by the presence of central obesity in these subjects. We have previously shown that sRAGE levels are inversely correlated with body mass index in a general population, irrespective of the presence or absence of hypertenstion [6,8]. Since RAGE expression levels were up-regulated during the differentiation process to mature adipocytes [9], adipocyte-derived sRAGE production may be decreased in patients with central obesity, which could partly explain the inverse association of sRAGE with circumference in our subjects. Anyway, markers of central obesity such as waist circumference may be a confounder of sRAGE and should be entered into the multivariate model when using sRAGE levels as a disease biomarker.

In this study, we found that sRAGE levels were inversely associated with Hb values. The present finding has extended the previous observation showing that sRAGE levels were independently associated with anemia in older community-dwelling women [10]. AGEs not only alter the deformability of red blood cells, but also promote the adhesion of erthrocytes to endothelial cells via the interaction with RAGE [11,12]. The AGE-RAGE interaction may influence the fragility or lifespan of red blood cells in NAFLD subjects.

5. Limitations

The major limitation of the present study is the lack of control group. It would be interesting to compare the data of NAFLD patients with those of age- and sex-matched controls and investigate the independent correlates of sRAGE in each group separately. Our study was a cross-sectional one and could not assess the questions of whether decreased sRAGE levels were a

Table 2. Univariate and stepwise multiple regression analyses for determinants of sRAGE

	Univariate*		Multivariate+		
Factors	β		P	β	P
Age	0.133	2.552	0.248		
Sex	-0.125	66.752	0.281		
Body mass index	-0.185	8.558	0.107		
Waist circumference (cm)	-0.269	3.583	P<0.05	-0.314	P<0.01
Hb (g/dL)	-0.330	20.685	P<0.01	-0.358	P<0.01
WBC (x10^3/mL)	-0.270	0.022	P<0.05		
Platelets (10^4/mL)	0.008	6.830	0.944		
AST (IU/L)	0.030	1.153	0.792		
ALT (IU/L)	-0.010	0.563	0.932		
γ-GTP (IU/L)	0.198	0.387	0.084		
Total-bilirubin (mg/dL)	-0.268	79.510	P<0.05		
Direct-bilirubin (mg/dL)	-0.195	304.058	0.089		
Albumin (g/dL)	-0.034	79.041	0.768		
T-Chol (mg/dl)	-0.084	0.826	0.467		
TG (mg/dl)	0.079	0.442	0.493		
HDL-C (mg/dl)	-0.019	2.385	0.871		
Uric acid (mg/dL)	0.048	23.703	0.676		
FFA (mEq/L)	-0.229	132.845	P<0.05		
Type IV collagen (ng/mL)	0.027	35.026	0.818		
Hyaluronic acid (ng/mL)	-0.159	0.284	0.171		
P-III-P (U/mL)	0.080	135.440	0.491		
Ferritin (ng/mL)	-0.255	0.241	P<0.05		
hsCRP (mg/L)	-0.118	60.097	0.307		
SFA (cm2)	-0.186	0.359	0.111		
VFA (cm2)	-0.042	0.718	0.718		
NAS	-0.116	20.529	0.317		
L/S density ratio	0.016	105.065	0.893		
HbA1c (%)	-0.247	37.708	P<0.05		
FPG (mg/dL)	-0.175	1.440	0.129		
HOMA-IR	0.085	2.965	0.465		
Insulinogenic index	-0.009	48.752	0.951		

**Univariate coefficients. β: Regression coefficients.*

+A stepwise multivariate regression analysis was performed.

R^2=0.176

sRAGE; soluble form of receptor for advanced glycation end products, Hb; hemoglobin, WBC; white blood cells, AST; aspartate aminotransferase, ALT; alanine aminotransferase, γ-GTP; γ-glutamyl transpeptidase, T-Chol; total cholesterol, TG; triglycerides, HDL-C; high-density lipoprotein cholesterol, FFA; free fatty acid, P-III-P; procollagen III N-terminal peptide, hsCRP; high-sensitivity C-reactive protein, SFA; subcutaneous fat areas, VFA; visceral fat areas, NAS; nonalcoholic fatty liver disease activity score, L/S; liver to spleen, HbA1c; glycated hemoglobin, FPG; fasting plasma glucose, HOMA-IR; homeostasis model assessment of insulin resistance.

cause or consequence of central obesity in NAFLD patients. Moreover, clinical significance of serum levels of sRAGE in low Hb values remains to be clarified. Future longitudinal and/or interventional studies are needed to solve these issues.

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