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Positive association of serum levels of advanced glycation end products and high mobility group box–1 with asymmetric dimethylarginine in nondiabetic chronic kidney disease patients

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Abstract

There is accumulating evidence that engagement of the receptor for advanced glycation end products (RAGE) with ligands such as advanced glycation end products (AGEs) and high mobility group box–1 (HMGB-1) elicits vascular inflammation, thus contributing to the increased risk for cardiovascular disease. Furthermore, enhanced accumulation of asymmetric dimethylarginine (ADMA) plays a role in cardiovascular disease in chronic kidney disease (CKD) patients. However, the relationships among serum levels of AGEs, HMGB-1, soluble form of RAGE (sRAGE), and ADMA are largely unknown. The aim of the present study is to determine their relationships in CKD patients. Twenty nondiabetic normotensive CKD patients with dyslipidemia and 20 age- and sex-matched healthy controls were enrolled. All subjects underwent determination of blood chemistries; urinary proteinuria; and serum levels of AGEs, HMGB-1, sRAGE, and ADMA. Serum AGE, HMGB-1, sRAGE, and ADMA levels in CKD patients were significantly higher than those in control subjects. Circulating levels of AGEs in CKD patients were positively associated with sRAGE and ADMA, and HMGB-1 with ADMA, but not sRAGE. There were no significant associations among these markers and serum creatinine, estimated glomerular filtration rate, proteinuria, and lipid levels. In multiple regression analyses, AGEs and HMGB-1 were independently correlated with ADMA. The present study demonstrated that AGE and sRAGE levels were correlated with each other and that AGEs and HMGB-1 were independently associated with ADMA in nondiabetic CKD patients. Elevation of the RAGE ligands may enhance ADMA levels, suggesting the active involvement of AGE/HMGB-1–RAGE–ADMA axis in CKD patients.

1. Introduction

Endothelial dysfunction with reduced nitric oxide production and/or bioavailability is a common feature in patients with apparent coronary atherosclerosis or in high risk-patients with chronic kidney disease (CKD) or diabetes, thereby contributing to the development and progression of cardiovascular disease (CVD) [1,2]. Because asymmetric dimethylarginine (ADMA) is an endogenous nitric oxide synthase inhibitor and its levels are increased in CKD patients [3,4], it is considered that ADMA is a novel emerging risk factor for CVD in patients with CKD. However, the underlying molecular mechanisms for the elevation of ADMA in CKD patients are not fully understood.

Receptor for advanced glycation end products (RAGE) is a member of the immunoglobulin superfamily of cell surface

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molecules capable of interacting with a broad spectrum of ligands including a diverse group of reducing sugar complexes with proteins, lipids, and nucleic acids [5]. Recent studies have shown that engagement of RAGE with ligands such as advanced glycation end products (AGEs) and high mobility group box–1 protein (HMGB-1) elicits oxidative stress generation and subsequently evokes inflammatory and thrombogenic responses in various types of cells, thereby playing an important role in diabetic accelerated atherosclerosis [5-7]. In addition, there are several articles showing that serum levels of AGEs, HMGB-1, and soluble form of RAGE (sRAGE) are elevated in diabetic patients, especially those with coronary artery disease [8-13], thus suggesting that they are novel biomarkers for CVD in diabetes.

Advanced glycation end product–modified proteins are shown to inhibit the enzymatic activity of dimethylarginine dimethylaminohydrolase (DDAH), an enzyme that mainly degrades ADMA in vivo, in cultured endothelial cells [1,2,14]. Furthermore, DDAH activity in endothelial cells is suppressed under oxidative stress conditions [15]. These findings led us to speculate that the activation of RAGE system with ligands such as AGEs and HMGB-1 could be involved in the ADMA elevation in patients with CKD. In this study, we measured serum levels of AGEs, HMGB-1, sRAGE, and ADMA simultaneously and investigated their relationships in early-stage CKD patients without diabetes.

2. Subjects and methods

2.1. Subjects

Twenty nondiabetic stage 1 or 2 CKD patients with dyslipidemia (total cholesterol [T-chol] >220 mg/dL, low-density lipoprotein cholesterol [LDL-C] >140 mg/dL, 150 < triglyceride [TG] < 400 mg/dL, or high-density lipoprotein-cholesterol [HDL-C] <40 mg/dL) (14 men and 6 women; immunoglobulin A nephropathy, n = 13; non–immunoglobulin A type proliferative glomerulonephritis, n = 5; membranous nephropathy, n = 2; mean age, 35.7 ± 5.8 years) and 20 age- and sex-matched healthy controls (14 men and 6 women; mean age, 37.2 ± 6.4 years) were enrolled in the present study. All patients were normotensive (blood pressure <130/80 mm Hg); and none of them received antihypertensive or antihyperlipidemic drugs such as angiotensin-converting enzyme inhibitors, angiotensin II type 1 receptor blockers, and statins. We excluded any patients with chronic pulmonary diseases, liver diseases, and neoplastic disorders and those who had recent (<6 months) acute coronary syndromes, stroke, and any acute infections. Patients who were younger than 20 years old, whose serum creatinine (Cr) level was more than 1.5 mg/dL, or whose proteinuria was more than 3.0 g/d were also excluded. Initially, 24 nondiabetic CKD patients were enrolled; but 4 patients were excluded because of the presence of massive proteinuria (n = 2) or liver diseases (n = 2). The study protocol was approved by the local ethical committee of Shinmatsudo Central General Hospital, and informed consent was obtained from all study participants. The study complied with the principles of the Helsinki Declaration.

2.2. Data collection

Blood pressure was measured in the sitting position twice after 2 minutes of rest using an upright standard sphygmomanometer. Mean value of blood pressures was used for analysis. Renal function was evaluated by serum Cr levels and estimated glomerular filtration rate (eGFR) according to the Modification of Diet in Renal Disease equation modified for the Japanese population [16]. Serum levels of T-chol, TG, and HDL-C were measured enzymatically at Shinmatsudo Central General Hospital. Low-density lipoprotein cholesterol level was calculated using the Friedewald formula. Serum ADMA level was analyzed by a high-performance liquid chromatography as described previously [17]. Serum levels of AGEs, sRAGE, and HMGB-1 were measured with enzyme-linked immunosorbent assays (ELISAs) as described previously [18-22]. In this study, 1 U of AGEs corresponds to 1 μg of glyceraldehyde-derived AGE–bovine serum albumin as described previously [19]. Intraassay and interassay coefficients of variation of sRAGE ELISA were 7.7% and 5.7, respectively. Intraassay and interassay coefficients of variation of HMGB-1 ELISA were less than 10%, and the detection limit was 0.3 ng/mL [21,22].

2.3. Statistical methods

Data were expressed as mean ± standard deviation. To compare the parameters between CKD patients (n = 20) and healthy controls (n = 20), we used the Wilcoxon signed-rank test. Correlations among serum AGEs, HMGB-1, sRAGE, and ADMA and clinical variables were determined by a

Table 1
Characteristics of CKD patients and age- and sex-matched healthy controls

	CKD patients	Healthy controls	*P* value
Age (y)	36.7 ± 5.8	37.2 ± 6.4	*P* = .699
Sex (male/female)	14/6	14/6	
SBP (mm Hg)	126.7 ± 7.3	122.6 ± 6.2	*P* = .521
DBP (mm Hg)	76.7 ± 4.3	73.4 ± 3.6	*P* = .642
T-chol (mg/dL)	244.7 ± 12.5	162.8 ± 14.0	*P* < .001
LDL-C (mg/dL)	172.6 ± 13.0	102.7 ± 10.5	*P* < .001
HDL-C (mg/dL)	39.1 ± 3.0	59.5 ± 5.8	*P* < .001
TG (mg/dL)	165.7 ± 11.9	102.7 ± 10.5	*P* < .001
Cr (mg/dL)	0.763 ± 0.053	0.709 ± 0.072	*P* < .05
eGFR (mL/min)	86.58 ± 10.43	92.9 ± 10.7	*P* = .062
Proteinuria (g/d)	1.15 ± 0.20	0	
ADMA (nmol/mL)	0.621 ± 0.050	0.361 ± 0.048	*P* < .001
HMGB-1 (ng/mL)	1.20 ± 0.30	ND	
AGEs (U/mL)	13.24 ± 1.33	6.3 ± 1.7	*P* < .001
sRAGE (pg/mL)	1244.0 ± 84.9	506.3 ± 132.2	*P* < .001

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; ND, not detected.

linear regression analysis. To determine independent determinants of serum ADMA levels, multiple stepwise linear regression analysis was performed. Statistical significance was defined as $P < .05$. All statistical analyses were performed with the use of the SAS system (SAS Institute, Cary, NC).

3. Results

Background of the patients is shown in Table 1. All patients were normotensive and dyslipidemic. Total cholesterol, LDL-C, and TG levels were significantly higher and HDL-C levels were significantly lower in CKD patients compared with those in healthy controls ($P < .0001$). Serum Cr levels in CKD patients were slightly higher than those in healthy controls ($P < .05$), but there was no significant difference of eGFR levels between the 2 groups. Serum AGE, sRAGE, and ADMA levels were significantly elevated in CKD patients compared with those in healthy controls ($P < .001$). Serum HMGB-1 levels were 1.20 ± 0.30 ng/mL in CKD patients, but it was not detected with our ELISA system in healthy controls (<0.3 ng/mL).

In CKD patients, circulating AGE levels were positively associated with serum levels of sRAGE ($r = 0.842$, $P < .001$) and ADMA ($r = 0.575$, $P = .008$), but not HMGB-1 ($r = 0.075$, $P = .753$) (Fig. 1). Furthermore, serum levels of HMGB-1 were positively correlated with ADMA ($r = 0.448$, $P = .048$), but not sRAGE ($r = -0.116$, $P = .626$) (Fig. 2). Because the parameters could be closely correlated with each other, to determine independent determinants of serum ADMA levels, multiple stepwise regression analysis was performed. This analysis showed that AGEs ($P = .006$) and

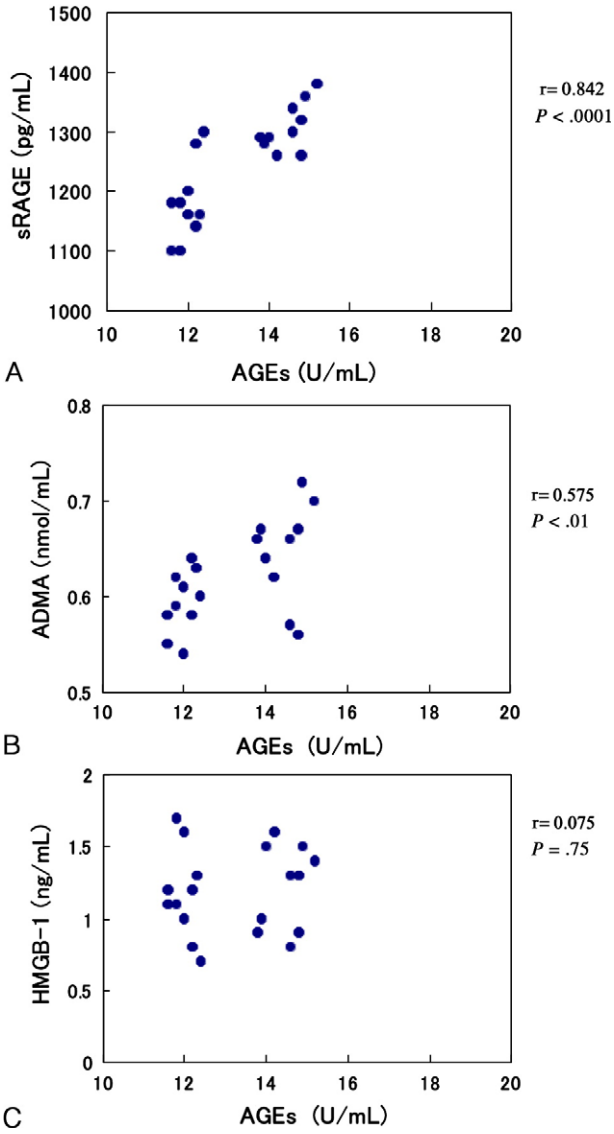

Fig. 1. Correlations between serum levels of AGEs and sRAGE (A), ADMA (B), and HMGB-1 (C) levels in CKD patients.

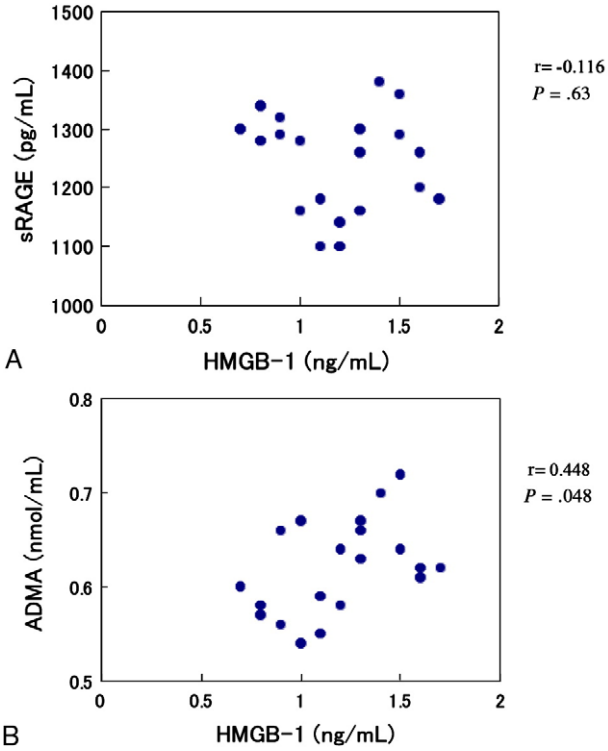

Fig. 2. Correlations between serum levels of HMGB-1 and sRAGE (A) and ADMA (B) in CKD patients.

Table 2
Multiple stepwise regression analysis for determinants of serum ADMA levels in CKD patients

Parameters	β	SE	P value
AGEs	.544	0.006	.006
HMGB-1	.407	0.029	.031
sRAGE	.183	0.001	.605

$R^2 = 0.435$. β indicates standardized regression coefficients; SE, standard error.

HMGB-1 (P = .031) were independently related to ADMA levels (R^2 = 0.435) (Table 2). There were no significant associations among all the parameters assayed here and T-chol, LDL-C, HDL-C, TG, Cr, eGFR, and proteinuria levels in both CKD patients and control subjects.

4. Discussion

In the present study, we demonstrated for the first time that serum levels of AGEs and sRAGE were correlated with each other and that AGE and HMGB-1 levels were independently associated with ADMA in nondiabetic early-stage CKD patients. This study has extended our previous findings that serum AGE levels were positively correlated with sRAGE and that their levels were associated with inflammatory markers and coronary artery disease in type 2 diabetes mellitus patients [9-11]. Although exogenously administered sRAGE was shown to exert atheroprotective properties in diabetic apolipoprotein E–null animals by acting as a decoy receptor for AGEs [23-25], it is questionable that sRAGE in humans could also exert the same biological effects because human serum levels of sRAGE are 1000 times lower than needed for the binding to AGEs [12,15,25]. Therefore, our present findings further support the concept that endogenous sRAGE could not efficiently capture and eliminate circulating AGEs in humans. Recently, most of the sRAGE in human blood has been found to be generated from the cleavage of cell surface RAGE by the action of sheddase, a disintegrin and metalloproteinase 10 [26]. Because AGEs up-regulate tissue RAGE expression [27-30], sRAGE levels in human blood may reflect tissue RAGE expression and ongoing inflammation and be elevated in response to circulating AGEs as a countersystem against the AGE-elicited inflammation in nondiabetic CKD patients.

In this study, serum levels of HMGB-1 were not correlated with sRAGE. This finding was also consistent with the recent observations in patients with diabetes showing that increased serum HMGB-1 levels were associated with coronary artery disease, but not with sRAGE [15]. These observations suggest that the kinetics and regulation of serum AGEs and HMGB-1 could differ. The differences of serum concentrations and binding affinity to RAGE between the 2 could account for the different correlations of these factors with sRAGE [31,32]. Compared with HMGB-1, circulating AGEs may be a stronger stimulant for RAGE expression and subsequent sRAGE generation in humans.

In the present study, we found for the first time that serum levels of AGEs and HMGB-1 were elevated in nondiabetic early-stage CKD patients. Although serum AGE and HMGB-1 levels were reported to increase in diabetic and/or stage 3 to 5 CKD patients [15,33], it is unlikely that blood glucose levels or renal function could affect our present results because (*a*) we enrolled nondiabetic stage 1 or 2 CKD patients without apparently active inflammatory diseases and (*b*) parameters associated with renal dysfunction such as serum Cr, eGFR, and proteinuria levels were not correlated with AGE or HMGB-1 levels. Because serum levels of high-sensitive C-reactive protein and oxidative stress markers are elevated in stage 1 or 2 CKD patients [3,4], increased oxidative stress generation and subclinical inflammation could contribute to the elevation of AGEs and HMGB-1 in our patients.

We also showed here first that circulating levels of AGEs and HMGB-1, but not sRAGE, were independent determinants of serum ADMA levels. Asymmetric dimethylarginine is mainly degraded by DDAH, whose enzymatic activity is suppressed by oxidative stress [15]. Therefore, it is conceivable that the AGE/HMGB-1–RAGE interaction elicits oxidative stress generation and subsequently inactivates DDAH activity, thus leading to increased ADMA production by various types of cells. Therefore, our present findings suggest that the activation of the AGE/HMGB-1–RAGE axis is involved in the elevation of ADMA, which could partly explain the increased risk for CVD in nondiabetic stage 1 or 2 CKD patients [34].

5. Limitations

First, our study was a cross-sectional one and, therefore, does not elucidate the causal relationships among serum AGE, HMGB-1, sRAGE, and ADMA levels. Therefore, we do not know whether circulating levels of AGEs or HMGB-1 could be mechanistically related to vascular inflammation and ADMA elevation. In vitro or in vivo testing in animal models could clarify the causal relationships among these factors and provide the mechanistic insight into how the factors were correlated with each other. Second, further study is needed to strengthen the concept that serum sRAGE is a proteolytically cleaved form from cell surface RAGE that is shed into the bloodstream and therefore serves as a marker for vascular injury in vivo. For this, examining a direct link between serum sRAGE and RAGE expression levels on peripheral blood mononuclear cells or vascular wall cells in patients with nondiabetic CKD would be helpful in a future study. Third, unfortunately, oxidative stress markers were not measured in our subjects because of the lack of serum samples. Therefore, whether oxidative stress markers are correlated with AGEs, HMGB-1, sRAGE, and ADMA in nondiabetic stage 1 or 2 CKD patients remains unclear. Interventional studies with antioxidants would be helpful to clarify whether oxidative stress is involved in positive correlations among these factors.

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