Original Article

Association of Serum Soluble Receptor for Advanced Glycation End Products (sRAGE), S100A12 and Pulse Pressure Value in Coronary Artery Disease Patients

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ABSTRACT

Objective: Coronary artery disease (CAD) causes more than 1 million deaths in China annually. The incidence of coronary artery disease tends to increase in the middle age and elderly people. Pulse pressure is associated with increased risk of CAD in middle-aged and older subjects, whereas soluble receptor for advanced glycation end products (sRAGE) and S100A12 are involved in pathology of atherosclerosis. In this study, we examine the associations of the expression level of sRAGE, S100A12 and PP value in both CAD and non-CAD groups; thus, providing more comprehensive strategies on preventing CAD. **Method**: Selected were divided into two main groups based on their angiograph results: CAD group (patients with at least one coronary artery of more than 50% occlusion) and Non-CAD Group (patients with less than 50% occlusion or without coronary artery occlusion). Soluble RAGE (sRAGE) and S100A12 concentration in blood samples were collected before CAG procedure were measured using ELISA. Pulse Pressure was measure from the difference between SBP and DBP. **Results:** Significant decrease in concentration of serum sRAGE ($p<0.05$) and S100A12 ($p<0.01$) was observed, while PP value ($p<0.01$) had significantly higher level in patients with CAD. The value of PP ($r = 0.35$, $p < 0.01$), expression level of both sRAGE ($r = -0.21$, $p < 0.01$) and S100A12 ($r = -0.24$, $p < 0.01$) had association with prevalence of CAD. **Conclusion**: Low level of sRAGE expression and higher pulse pressure in CAD group are associated to coronary artery disease within middle age and elderly people of the Chinese population. Therefore providing alternative novel therapeutic strategies may prevent CAD before entering the middle age.
Key words: Serum soluble receptor for advanced glycation end products (sRAGE), S100A12, Pulse Pressure, Coronary Artery Disease (CAD).

INTRODUCTION

Coronary artery disease (CAD) is the most common type of heart disease. Every year coronary artery disease causes more than 1 million deaths in China.[1] CAD risk factors such as hypertension, diabetes, smoking and obesity are modifiable.[2] However, the incidence of coronary artery disease also has increased in the middle age people and elderly.[3]

There is increasing evidence that Pulse Pressure (PP), in middle-aged and older subjects, is associated with increased risk of cardiovascular disease and predicts future cardiovascular events in patients with CAD.[4] PP calculated from the difference between systolic blood pressure (SBP) and diastolic blood pressure (DBP), can be use as indicators for increased large arterial stiffness.[5] Geroldi *et al* found that there is a strong association between soluble receptor for advanced glycation end products sRAGE and PP. Their study suggests that serum sRAGE levels are low in patients with arterial stiffness.[6]

Assessment of Arterial stiffness through PP can provide additive value to detect the presence of CAD and facilitate cardiovascular risk stratification beyond traditional risk factors.[7] In addition, expression level of sRAGE may demonstrate pathophysiological mechanisms through which PP may affects progression of atherosclerosis.

Past studies revealed that sRAGE may reflect enhanced activity in the RAGE system due to the effects of ligand stimulation mediate sRAGE upregulation[8-9] and sRAGE is secreted in parallel with RAGE.[10-11] Previous studies have shown that sRAGE acts as a decoy receptor for RAGE ligands. Reduced levels of sRAGE are thought to be an expression of an impaired protection against inflammation and cell injury.[12-13] RAGE

ligand such as S100A12 has played a significant role in the pathogenesis of atherosclerosis through RAGE binding ligand reaction.[14]

Circulating S100A12 is associated with inflammatory markers and may reflect an individual's disease progression.[15-16] Therefore it is important to assess clinical association among serum level of S100A12, sRAGE and PP value, as a result of which may establish better basis for further medical assessment which will prevent development of CAD among middle age and elderly people.

MATERIALS AND METHODS

Study Population

The study protocol followed the guidelines of the local ethics committee and all participants provided their informed consent to be part in this analysis. The study population consisted of 175 subjects (98 men and 77 women, 62.3 ± 7.9 years) who were recruited during July 2016 to December 2016 from the Jiangsu Provincial Hospital which is affiliated to Nanjing Medical University, where individuals were referred by general practitioners for elective coronary angiography.

The study classified the sample size into two main groups; Group 1 consisted of 99 angiographically documented CAD patients who diagnosed as having CAD with organic coronary artery stenosis (≥50%) and Gensini scores were examined for evaluation of severity of CAD,[17] Group 2 consisted of 76 angiographically documented CAD patients termed as Non-CAD patients who have organic coronary artery stenosis (<50%) or with no occluded coronary artery.

Indications for elective angiography included patients with both stable and unstable angina, patients with present CAD, patients with nonspecific

chest pain, preoperative evaluation of patients with valvular heart disease.[18] Patients with several conditions are excluded from the sampling. The conditions were as follow: any type of malignancies, liver and/or kidney dysfunction, thyroid disease, acute coronary syndrome, acute or chronic inflammatory disease, autoimmune, cardiomyopathy, acute cerebrovascular and peripheral vascular diseases, peripheral artery disease.

The detailed medical record for each selected patient (including family history, past and present illnesses) was compiled and routine physical examinations and laboratory tests were carried out.

Anthropometric parameters (height and body weight) were recorded using a standard protocol. Body Mass Index (BMI) was calculated as weight in kilograms divided by the square of the height in meters.

Both systolic and diastolic blood pressure of each patient was measured with a mercury sphygmomanometer after a minimum of 10-min rest in the sitting position. Pulse pressure was calculated as the difference between SBP and DBP.

The study also took note of cardiovascular risk factors such as smoking habits, hypertension and diabetes mellitus. Out of the 175 patients selected, 56 patients were smokers, 92 had hypertension and 38 had diabetes mellitus.

Blood sample collection

Blood sample collections were collected twice: once in the morning prior to the operation and the angiography session. The first blood collection extracted 7ml of venous blood in the morning before the operation and after a 12-h overnight fast and is mainly used for baseline blood profile analysis. These samples were drawn from the antecubital vein. Samples were drawn and tested in accordance with standard hospital procedure in Jiangsu Province Hospital.

The second blood collection was carried out before angiography session, Blood was collected through a guiding sheath and were mainly used for measuring serum level of sRAGE and S100A12. Blood samples of each patient were centrifuged at 3000 rpm for 10 min at 4°C and stored at –59°C in order to separate serums from cells. Serum sRAGE and S100A12 concentration were measured by an enzyme-linked immunosorbent assay (ELISA) kit method (Nanjing SenBeiJia Biological Technology Co., Ltd.)

Statistical analysis

All data is presented as mean±standard deviation or percentages and was analyzed with SPSS 14.0 (SPSS, Inc., Chicago, IL, USA). Mean levels of different groups were compared by independent t-test. To compare the participants' characteristics, we used the chi-square test for categorical variables. Correlations between variables were analyzed with Pearson's coefficient. Binary logistic regression analysis was used to determine the association between the groups and all other variables considered in the present study. All tests for statistical significance were two-sided and $P<0.05$ was defined as statistically significant.

RESULTS

Measurement of baseline characteristics in all patients

There were no significant differences in age, gender, BMI, heart rate, smoking history, diastolic blood pressure and Ejection Fraction between 2 groups of patients. Nevertheless, it is observed that there is a significant difference in the PP ($P<0.01$) and SBP ($P<0.05$) between CAD and Non-CAD groups. Group 1 shows that PP and SBP is higher than group 2 (refer to Table 1). These results also denote that major cardiovascular risk factors, such as hypertension ($P<0.01$) and diabetes mellitus ($P<0.01$), has significant differences between two groups. Group 1 has higher number of diabetes and hypertensive patients than Group 2.

Table 1: Baseline characteristics of all the study subjects.

	Group 1 - CAD (n=99)	Group 2 - non CAD (n=76)	*P* value
Demographic indexes			
Age (years)	62.9 ± 9.6	61.5 ± 8.8	P = 0.19
Male/female	55/44	43/33	P = 0.89
BMI (kg/m2)	24.3 ± 3.0	24.1 ± 3.6	P = 0.74
Heart Rate (bpm)	76.1 ± 15	78.9 ± 16.4	P = 0.24
Clinical indexes			
Hypertension (%)	65 (65.7)	27 (35.5)	P < 0.01
Diabetes mellistus (%)	29 (29.3)	9 (11.8)	P < 0.01
Smoking (%)	33 (33.3)	23 (30.3)	P = 0.67
SBP (mmHg)	129.7 ± 17.5	118.7 ± 17.1	P < 0.01
DBP (mmHg)	73.8 ± 16.2	70.11 ± 15.1	P = 0.61
PP (mmHg)	55.5 ± 16.7	44.2 ± 13.7	P < 0.01
EF (%)	61.3 ± 8.1	59.7 ± 7.1	P = 0.17
Blood measurement			
Hemoglobin (g/l)	122.4 ± 21.3	118.6 ± 18.6	P = 0.218
Platelet (10^9/L)	203.1 ± 79.2	186.9 ± 83.4	P = 0.193
D-Dimer (µg/mL)	1.27 ± 2.2	1.31 ± 1.82	P = 0.914
Fibrinogen (mmol/l)	2.6 ± 1.06	2.5 ± 0.93	P = 0.36
TC (mmol/l)	4.1 ± 1.58	4.1 ± 1.1	P = 0.646
HDL- C (mmol/l)	1.1 ± 0.47	1.1 ± 0.73	P = 0.312
LDL- C (mmol/l)	2.5 ± 0.86	2.6 ± 0.82	P = 0.303
TG (mmol/l)	1.8 ± 4.7	1.3 ± 1.1	P = 0.437
FPG (mmol/l)	6.2 ± 1.6	6.0 ± 2.2	P = 0.61
UA acid (mmol/l)	312.9 ± 88.7	334 ± 117.1	P = 0.176
Serum biomarkers			
S100A12 (ng/L)	232.0 ± 75.9	299.5 ± 191.6	P < 0.01
sRAGE (ng/L)	41.36 ± 32.49	57.88 ± 45.36	P < 0.05

Hb, hemoglobin; D-D, d-dimer; EF, Ejection Fraction; DBP, Diastolic Blood Pressure; SBP, Systolic Blood Pressure; PP, Pulse Pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; FGP, fasting plasma glucose; UA, uric acid.

Measurement of blood profile levels in all patients

Blood profiles of all sample population are presented in Table 2. Between the two groups, it is observed that there is no significant difference in serum levels: TC, HDL-C, LDL-C, TG, Hb, FPG and UA. Blood coagulation profiles platelet, FIB and D-D between two groups do not have significant difference either.

Expression levels of sRAGE and S100A12 in arterial.

To investigate the expression levels of sRAGE and S100A12 in arterial blood, immunohistochemistry was performed. As shown in Table 1, the average expression level of sRAGE was 41.36 ± 32.49 ng/L and the average expression level of S100A12 is 232.0 ± 75.9 µg/L in the CAD patients. While in non-CAD patients, the average expression level of sRAGE is 57.88 ± 45.36 ng/L and the average expression level of S100A12 is 299.5 ± 191.6 µg/L. Significant decrease in expression level of serum sRAGE ($P< 0.05$) and S100A12 ($P< 0.01$) in group 1 is observed.

Table 2: Associations of serum sRAGE, S100A12, PP and coronary artery disease as determined by Pearson's correlation analysis.

Variable	CAD group	Pulse Pressure	S100A12	sRAGE
CAD group	-	0.345 **	-0.236 **	-0.207 **
PP	-	-	-0.354	-0.301 **
S100A12	-	-	-	0.72 **
sRAGE	-	-	-	-

*** p < 0.01*

Figure 1: Scatter plot between serum sRAGE and S100A12 in both groups.

Figure 2: Scatter plot between serum sRAGE and Pulse Pressure in both groups.

Correlational analysis was conducted to investigate the relationship among laboratory measures of variables from cohorts of CAD and Non-CAD with both groups. The results are presented in Table 2.

The value of PP ($r = 0.345$, $p < 0.01$), expression level of both sRAGE ($r = -0.207$, $p < 0.01$) and S100A12 ($r = -0.236$, $p < 0.01$) had association with prevalence of CAD. PP value were inversely related to serum sRAGE levels ($r = -0.301$, $p < 0.01$) for both groups. While serum sRAGE levels were positively related to serum S100A12 level ($r = 0.72$, $p < 0.01$) in our study group.

Further, logistic regression analysis revealed that PP, sRAGE and S100A12 statistically were potential predictors of among CAD group. These three variables cumulatively demonstrated a sensitivity (82.5%) and specificity (88%).

DISCUSSION

In the present study, CAD patients have higher prevalence of hypertension and diabetes than non-CAD group. In some studies about prevalence of cardiovascular disease (CVD) risk factor in Chinese population, hypertension seems to be the most important factor.[19,20] Although Diabetes prevalence has significant difference, both CAD and Non-CAD group have shared similar glycemic state (FPG) during the measurement, leaving hypertension ($P<0.01$, $r = 0.229$) as a significant CAD risk factor influencing our study. It is to be considered, since the there is significant difference between SBP ($p <0.01$) between 2 groups.

Blood pressure which defines hypertension has 3 important components: systolic (SBP), diastolic (DBP) or pulse pressure (PP).[21] Characteristic of SBP tends to increase progressively with age and in the ageing societies, elevated SBP is the most common form of hypertension.[22] The progressive changes of systolic and diastolic blood pressure with age lead to elevation in PP,[21] which has emerged potentially independent risk factors for CVD itself.

Some studies also have shown that PP is potential for predicting cardiovascular outcomes among middle age and elderly patients.[23-26] As the range of ages in our study is between middle age and elderly,[27-29] we had found that CAD group had higher PP comparing to the Non-CAD group. Higher PP reflects increased central arterial stiffness that in turn increases SBP.[30] There was no significant difference of DBP between 2 groups, while CAD patients have significantly higher SBP comparing to non-CAD patients. Although we did not perform specific measurement comparing arterial stiffness between 2 groups, we cannot exclude the possibility that structural changes, including fragmentation of elastin, increased amount of collagen, arterial calcification, glycation of elastin, collagen and crosslinking of collagen with advanced glycation end products (AGEs) leading to the stiffness of the artery.[31-32] RAGE – its ligand intermolecular covalent bond or cross-linking can alter the characteristics of collagen.[33] AGEs–RAGE interaction may propagate inflammatory response that may be involved in the further pathogenesis of atherosclerosis.[34] Since sRAGE and esRAGE have protective effects against deleterious effects of interaction of AGEs with RAGE, they act as a decoy for RAGE by binding with RAGE ligand.[35] sRAGE is an isoform of RAGE, which lacks the intracellular domain and thus intracellular signalling.[36] Soluble RAGE (sRAGE) is produced in two different pathways, either endogenous secreted as a splice variant, esRAGE[37] or as a cleaved variant.[38] Our study found that sRAGE expression level in CAD patients is significantly lower than non-CAD patients. It corresponds with Colomba *et al.*[39] low levels of sRAGE in plasma are associated with the presence of CAD.

There are also past studies that revealed sRAGE may reflect enhanced activity in the RAGE system since the effects of ligand stimulation mediate sRAGE upregulation[40] and sRAGE is secreted in parallel with
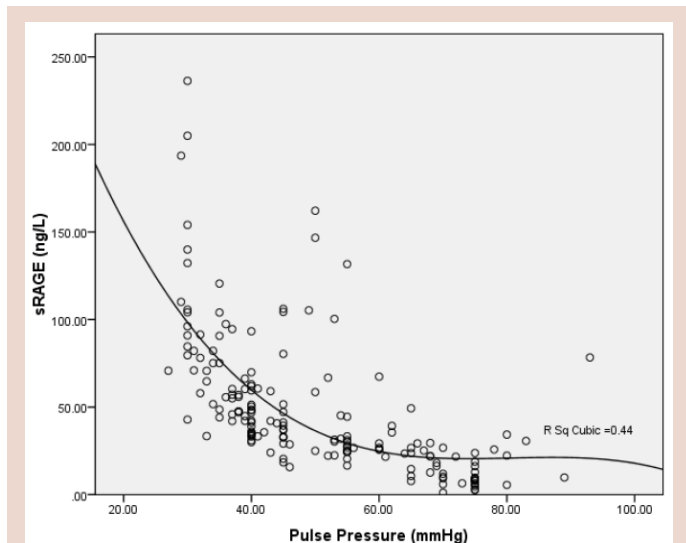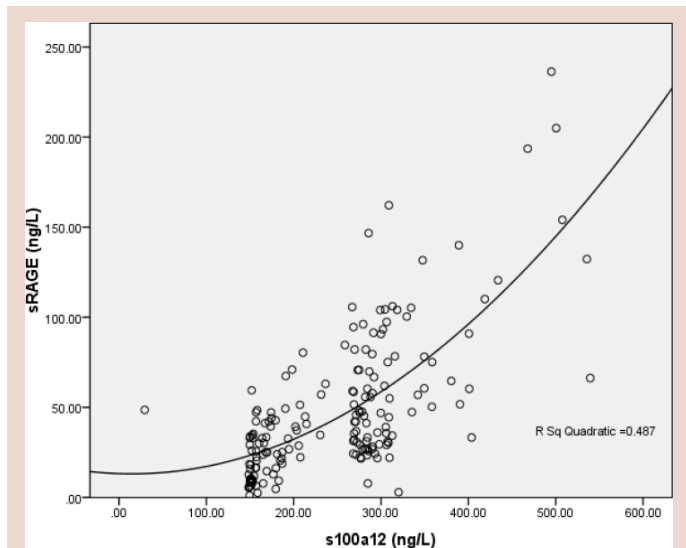
RAGE.[37-38] As RAGE levels in plasma is higher in CAD patients,[41] sRAGE should be higher as well, but this is in contradiction in our study. It might be caused by the binding of sRAGE and s100A12, reflecting less expression level of S100A12 in the CAD group. S100A12 is an inflammatory ligand of RAGE which regulates inflammation and immune response.[42] Its ligation with RAGE on the endothelium, mononuclear phagocytes and lymphocytes triggers cellular activation with the generation of key proinflammatory mediators in turn further progress the pathogenesis of atherosclerosis.[43] Therefore, the role of S100A12 in the pathogenesis among CAD patients has been known. While our findings do not show CAD group has higher expression level of S100A12. Another plausible explanation would be the sample population health profile. One study also showed that a serum S100A12 concentration is higher in patients with ACS than patients with stable CAD.[44] Since subjects in this study are mostly patients with stable CAD, level of S100A12 may not responded as sensitive.

Past studies had observed negative association between sRAGE levels and PP.[6] Since increase in PP has been related to coronary heart disease,[45-47] it is possible to speculate that low level of sRAGE expression and higher pulse pressure in CAD group are associated to coronary artery disease within Chinese population among middle age and elderly. The studies also suggest that controlling PP, elevation of sRAGE and exogenous administration of recombinant sRAGE may be novel therapeutic strategies in the prevention of CAD before entering the middle age.

LIMITATION

Several limitations of our study merited consideration. First, this study employs a cross-sectional study with a relatively small sample size. Further multi-centre prospective longitudinal studies on larger population are needed in order to reaffirm the observation. Second, most of our patients underwent coronary angiography; thus, factors such as hospitalization and lipid-lowering diets commonly adopted by CAD patients would affect serum indices. Third, our study is limited only for Chinese population.

ACKNOWLEDGEMENT

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

CAD: Coronary Artery Diseases; **sRAGE:** soluble advanced glycation end products; **CAG:** Coronary Angiography; **SBP:** systolic blood pressure; **DBP:** diastolic blood pressure; **PP:** Pulse Pressure; **BMI:** Body Mass Index; **Hb:** hemoglobin; **D-D**: D-dimer; **EF:** Ejection Fraction; **DBP:** Diastolic Blood Pressure; **SBP:** Systolic Blood Pressure; **TC:** Total Cholesterol; **HDL-C:** high-density lipoprotein cholesterol; **LDL-C:** Low-Density Lipoprotein Cholesterol; **TG:** triglycerides; **FGP:** Fasting Plasma Glucose; **UA:** uric acid; **CVD:** cardiovascular disease; **ACS:** Acute Coronary Syndrome.

SUMMARY

Since increase in PP has been related to coronary heart disease. Low level of sRAGE expression and higher pulse pressure in CAD group are associated to coronary artery disease within middle age and elderly people of the Chinese population. This proves that providing alternative novel therapeutic strategies may prevent CAD before entering the middle age.

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