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Serum levels of soluble receptor for advanced glycation end products (sRAGE) in Takayasu's arteritis

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Takayasu's arteritis (TA) is a rare, chronic inflammatory vasculitis that mainly affects the large elastic arteries, such as the aortic arch and its primary branches, leading to stenosis, occlusion, or aneurysm formation in vessels [1]. Despite predominantly affecting young persons, TA is associated with a significantly increased risk and a higher prevalence of atherosclerosis. Cardiovascular complications, including cardiac failure, pulmonary hypertension, cerebrovascular incident, myocardial infarction (MI), and ruptured aortic aneurysm are the major causes of morbidity and mortality [2]. In the natural course of TA, the patient experiences cycles of active and remission phases, that reflects the different inflammatory states of the arterial lesions [3].

Recently, we demonstrated increased oxidative stress and activity of Matrix metalloproteinases (MMPs) in perpetuation of inflammation in this disease [4]. The inappropriate activation of MMPs/or an imbalance between their action and those of tissue inhibitor (TIMPs) has been shown to influence the overall status of vascular remodeling. [5].

In the last few years, a growing number of studies have demonstrated that levels of soluble receptor for advanced glycation end products (sRAGE) decrease in the circulation in a range of diseases. We and others have demonstrated decreased levels of sRAGE in diseased conditions like diabetes mellitus, hypertension, hypercholesterolemia, non-diabetic coronary artery disease and Alzheimer's disease [6–13]. The receptor for advanced glycation end products (RAGE) is a multiligand receptor of the immunoglobulin superfamily that engages diverse ligands relevant to the pathogenesis of atherosclerosis. RAGE has a C-truncated secreted isoform, termed soluble RAGE (sRAGE). Differently from cell-surface RAGE, sRAGE blocks cell surface RAGE-ligand binding and subsequent signalling by acting as a decoy [14]. The status of sRAGE levels in subjects with TA in both active and remission phases is not yet known. Also, is there any correlation that exists between sRAGE and circulating levels of MMPs? To address these queries, we determined the serum levels of sRAGE, MMPs (1, 3 and 9) and TIMP-1 in subjects with TA and their control counterparts.

40 patients with angiographically proven Takayasu's arteritis and 40 normal healthy controls were enrolled in the present study as described earlier [4]. The study has been approved by The Institutional Ethics Committee.

Serum levels of MMP-1 (Amersham Biosciences, RPN 2610), MMP-3 (Amersham Biosciences, RPN 2613), MMP-9 (Amersham Biosciences, RPN 2614), TIMP-1 (Amersham Biosciences, RPN 2611) and sRAGE (R&D Sysytems, DRG00) were determined in all the study subjects using commercially available immunoassay kits. The immunoassay used for determination of soluble RAGE measures the total pool of soluble RAGE which is generated either by splicing or cleavage (e.g. sRAGE or esRAGE). Statistical analysis was performed using SPSS 17.0.

Anthropometric characteristics of all the study subjects are depicted in Table 1. We observed no significant difference between two study groups as far as lipid and lipoprotein profile, blood urea, serum creatinine and uric acid levels are concerned ($p>0.05$; data not shown). However, we observed a significant difference for MMPs, TIMP-1 and sRAGE in groups and sub-groups of study subjects (Table 1). We categorized TA subjects on the basis of absence and presence of hypertension at the time of enrollment in the study. We observed that except for sRAGE, all the other variables (MMP-1, 3, 9 and TIMP-1) were found to be insignificantly different between TA

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Table 1
Anthropometric characteristics of study subjects.

Investigations	Controls	TA	TA	
			Active disease	In remission
	(n = 40)	(n = 40)	(n = 32)	(n = 8)
Age range (years)	15–49	15–53	17–50	15–53
Sex ratio (M:F)	10:30	8:32	6:26	2:6
SBP (mm Hg)	117 ± 6	134*[a] ± 19	135 ± 19	128 ± 16
DBP (mm Hg)	76 ± 6	84*[a] ± 14	85 ± 14	80 ± 14
BMI (kg/m^2)	21.5 ± 3.1	20.7 ± 3.6	20.46 ± 3.0	21.4 ± 4.1
Smokers	5[$]	5[$]	5[$]	1[$]
Alcohol use	5[$]	6[$]	5[$]	1[$]
MMP-1 (ng/ml)	67.3 ± 16.8	65.2 ± 16.1	62.9 ± 11.1	74.5 ± 27.8
MMP-3 (ng/ml)	407.7 ± 98.5	438.8 ± 84.5	447.8 ± 52.4	402.5 ± 160.2
MMP-9 (ng/ml)	203.6 ± 123.7	290.2.2 ± 228.1.4*[a]	313.2.0 ± 234.5	197.9 ± 184.5
TIMP-1 (pg/ml)	367.8 ± 199.3	601.6 ± 288.1***[a]	615.1 ± 286.5	547.7 ± 308.3
sRAGE (pg/ml)	1710.8 ± 632.8	782.5 ± 518.3***[a]	574.9 ± 266.8	1612.8 ± 443.3***[b]

n = number of subjects; SBP-Systolic blood pressure; DBP—diastolic blood pressure; results are expressed as mean ± S.D; $ Occasional; AU = arbitrary units. [a]Takayasu's arteritis (TA) vs healthy controls (HC); [b]TA subjects studied during active phase (TAA) vs subjects studied during remission phase (TAR); ***p<0.001, *p<0.05.

patients who were hypertensive versus non-hypertensive. sRAGE levels were found to be significantly lower in TA subjects who were hypertensive (n = 10; 503.6 ± 243.0 pg/ml) as compared to the non-hypertensive (n = 30; 875.5 ± 554.2 pg/ml) (p<0.05). We observed no significant correlation of serum levels of sRAGE with age and any of the lipid and lipoproteins levels in TA subjects. However, sRAGE demonstrated a negative and significant correlation with MMP-3 (r = −0.223; p = 0.047) and MMP-9 (r = −0.278; p = 0.012) levels.

As MMP-9, TIMP-1 and sRAGE achieved significance (Table 1), ROC curves were constructed to determine the optimal values, which provide high sensitivity and specificity. The area under the curve (AUC) was determined which was found to be less for MMP-9 (AUC = 56.7%), high for TIMP-1 (AUC = 74%) and highest for sRAGE (AUC = 87.7%,). The sensitivity and specificity was calculated for each possible threshold value of estimated probability for the respective group. Cut-off value for sRAGE levels which achieved an optimal sensitivity of 92.5% and specificity of 72.5% was 889 pg/ml (Fig. 1).

Recent years have witnessed an increased attention to sRAGE as a biomarker and the present study represents a valid addition to this filed. This is the first clinical study to demonstrate that the serum levels of sRAGE are lower in TA patients as compared to their healthy control counterparts.

Matsuyama et al. [15] reported significantly augmented levels of MMP-9 and MMP-3 in TA subjects in active phase as compared to healthy controls (p<0.001). However, contrary to their observations, we observed no significant changes in these two MMP levels. However, our study is the first to report the levels of MMP-1 in these subjects, which similar to MMP-3 and 9 levels did not show any significant difference between controls versus TA subjects.

In an isolated report by Matsuyama et al. [15] for TIMP-1 levels in TA subjects, reported significantly high levels of TIMP-1 in controls as compared to those TA subjects who were with active disease. On the contrary, we observed significantly high levels of TIMP-1 in subjects with TA as compared to the controls (p<0.05). Further, TIMP-1 levels remained statistically higher in TA subjects in remission also when the data was compared with controls (p<0.05). Increased levels of TIMP-1 have been observed in patients with CAD [13,16] and in subjects with MI [17]. Higher levels of TIMP-1 in TA subjects in our study could be explained as a compensatory or adaptive phenomenon.

sRAGE has been documented as anti-atherogenic molecule, and a negative correlation of sRAGE with MMP-9 and MMP-3 provides further justification to the anti-atherogenic nature of this molecule. We also observed that TA subjects who were hypertensive had low levels of sRAGE as compared to those TA subjects who were normotensive. These observations are consistent with our previous

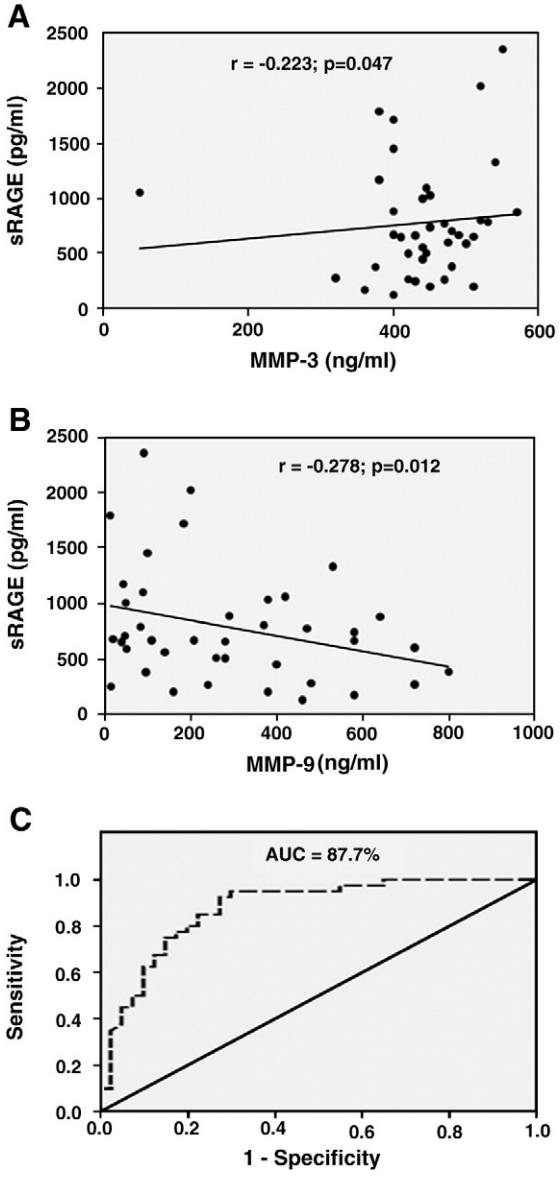

Fig. 1. Panels A and B: Scatter plots and linear Regression lines for correlation between sRAGE and MMP-3 and MMP-9 in subjects with Takayasu's arteritis; Panel C: Receiver operating characteristic (ROC) curve for sRAGE in study cohort.

findings in non-diabetic subjects with CAD [13] and that of Geroldi et al. [8], that levels of sRAGE were decreased in patients with hypertension and raised the possibility that sRAGE may play a role in arterial stiffness and its related complications. Recently proposed mechanisms for post-translational modifications of full length RAGE like shedding of ectodomain and proteolysis by MMP-9 or ADMA10 cannot be ignored [18,19]. However, we observed low levels of sRAGE in TA subjects, which may be explained as shedding may be modulated by binding of proinflammatory ligands or alternatively low levels of sRAGE may not be sufficient as a decoy for proinflammatory ligands and therefore do not protect against inflammation. We in the present study did not measure any inflammatory ligand in these study subjects, however, a similar negative relation has earlier been observed in one of our previous study with subjects in non-diabetic subjects with CAD [12].

In the present study, we observed a lack of correlation between BMI and sRAGE levels in TA subjects, which is supported by our earlier observations and also that of Geroldi et al. [10,13]. Moreover, sRAGE did not demonstrate any significant correlation with age in TA subjects. Contrary to our findings, Koyama et al. [11] in their study reported a significant inverse correlation of esRAGE and BMI in subjects with metabolic syndrome.

No study is without limitations and so is ours. Firstly, because of the cross-sectional design, causal relationship between low sRAGE concentration and TA and the mechanism by which plasma sRAGE levels are decreased in these subjects could not be determined. Unfortunately, we did not analyze the effects of medications on the sRAGE levels. Second, the patients enrolled in this study were only from the north Indian population, thus caution should be exercised for extrapolating the data to other ethnic groups. Lastly, the ELISA detection system used in the present study could not discriminate between specific soluble RAGE variants (sRAGE and esRAGE). Because of these important caveats, prospective longitudinal and intervening studies are required to confirm the role of sRAGE as a potential biomarker to be used in routine management for prediction of disease severity in patients with TA. Our study is an initial step in this direction.

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