Advanced Glycation End Products and its Soluble Receptors in the Pathogenesis of Thoracic Aortic Aneurysm

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Abstract

Background: Matrix metalloproteinases (MMPs) have been implicated in the pathogenesis of thoracic aortic aneurysms (TAAs). Cytokines [Interleukin (IL)-Iβ, IL-2, IL-6, and TNF-α)] increase the expression of MMP-2 and -3. Advanced glycation end products (AGEs) interact with cell receptors to increase the release of cytokines. Circulating soluble receptors for AGEs (sRAGE) and endogenous secretory RAGE (esRAGE) compete with membrane bound RAGE for binding with AGEs and reduce the production of cytokines. It is hypothesized that low levels of serum sRAGE and esRAGE and high levels of AGEs, AGEs/sRAGE, and AGEs/esRAGE would increase the levels of cytokines that would increase the levels MMPs, thus contributing to the formation of TAAs.

Methods: The study population was composed of 17 control subjects and 20 patients with TAA. Blood samples were collected for measurement of serum sRAGE, esRAGE, AGEs, cytokines, and MMPs. AGEs, sRAGE, and esRAGE were measured using ELISA kits, whereas the remaining parameters were measured using the Luminex Multi-Analyte system.

Results: The levels of sRAGE were lower, while the levels of AGEs, AGEs/sRAGE, AGEs/esRAGE, cytokines and MMPs were higher in patients with TAA compared to controls. The levels of sRAGE were inversely correlated with cytokines and MMPs, while AGEs, AGEs/sRAGE and AGEs/esRAGE were positively correlated with cytokines and MMPs. Cytokines were positively correlated with MMPs.

Conclusions: The data suggest that the AGE-RAGE axis may be involved in the pathogenesis of TAA and that low levels of sRAGE and high levels of AGEs, AGEs/sRAGE, and AGEs/esRAGE are risk factors for TAA.

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Introduction

The mechanism of development of thoracic aortic aneurysms (TAAs) is complex. The characteristic features of TAAs are destruction of collagen and elastin in media and adventitia, loss of smooth muscle cells with thinning of aortic wall, and transmural infiltration

of lymphocytes and macrophages. Matrix metalloproteinases (MMPs) -2 and -3, which have elastolytic and collagenolytic activities, have been implicated in the development of (aortic aneurysm) AA [1, 2]. Cytokines interleukin (IL)-1β and tumor necrosis factor-alpha (TNF-α) increase the expression of MMP-2. IL-1β [3, 4], IL-2 [5], IL-6 [6], and TNF-α [4] regulate the expression of MMP-2 and MMP-3.

Advanced glycation end products (AGEs) are a heterogeneous group of irreversible aducts resulting from nonenzymatic glycation and oxidation of proteins, nucleic acids and lipids [7, 8]. AGEs interact with receptors for AGEs (RAGE) and activate nuclear-factor kappa-B (NF-κB), increasing gene expression and releasing inflammatory cytokines (IL-1β, IL-2, IL-6, and TNF-α) [8–10] and generating reactive oxygen species (ROS) [8, 11]. There are two isoforms of c-truncated RAGE: total soluble RAGE (sRAGE) [12] and endogenous secretory RAGE (esRAGE) [13]. Both sRAGE and esRAGE act as a decoy for RAGE ligands and compete with membrane-bound RAGE for ligand binding [14], thus reducing the production of cytokines [3–6] and ROS [15]. Earlier, based on the literature, we [16] had suggested that sRAGE plasma levels may differentiate patients with aortic disease from the general population.

It is hypothesized that low levels of sRAGE and esRAGE and high levels of AGEs, AGEs/sRAGE, and AGEs/esRAGE would increase the levels of cytokines, which in turn would increase the levels of MMPs, resulting in the formation of aortic aneurysms. The specific objectives are to determine whether serum levels of sRAGE and esRAGE are lower, and AGEs, AGEs/sRAGE, AGEs/esRAGE, cytokines (IL-1β, IL-2, IL-6, TNF-α), and MMPs (MMP-2 and MMP-3) are higher in patients with TAA compared to control subjects. The other objectives are to determine if sRAGE and esRAGE are negatively correlated with cytokines and MMPs; if AGEs, AGEs/sRAGE, and AGEs/esRAGE are positively correlated with cytokines and MMPs; and if cytokines are positively correlated with MMPs.

Methods

Study Population

The study population was composed of 17 control and 20 patients with TAA. The control subjects were selected at the Royal University Hospital, University of Saskatchewan, Saskatoon, Canada. The following selection criteria for control subjects were used. They were in the age group of 35 to 50 years, nonobese, normotensive, nonsmokers, and had no history of angina, coronary artery disease, or diabetes. The patients with TAA were selected from Aortic Institute, Yale-New Haven Hospital, Yale University School of Medicine, (New Haven, Connecticut, USA) during the period of September 2011 to May 2012. The demographics and clinical characteristics of the patients with TAAs are shown in Table 1. The study protocol was approved by the Human Investigations Committee at Yale University, School of Medicine, New Haven, Connecticut, and the Ethics Committee for Human Studies at the University of Saskatchewan and by the Saskatoon Health Region. Written informed consent was obtained from control subjects and patients with TAA. TAA in figures has been represented as AA.

Measurements of Biochemical Parameters

Five milliliters of arterial/venous blood samples were collected from control subjects and intra-operatively from all patients undergoing surgery of thoracic aorta before heparin administration in a vacutainer without anticoagulant for measurement of serum sRAGE, esRAGE, AGEs, IL-Iβ, IL-2, IL-6, TNF-α, MMP-2, and MMP-3. Blood samples were immediately refrigerated at 4°C for 3 hours before centrifugation at 3000 rpm for 10 minutes at 4°C. The serum (supernatant) was collected and transferred into Eppendorf tubes and stored at −80°C until used for analysis. AGE levels in the serum were measured using a human AGE-enzyme-linked immunoassay (ELISA) kit (BioPCR, Beijing Zhonghao Shidai Co. Ltd. China). Serum levels of sRAGE and esRAGE were measured using the commercially available ELISA kit (R&D systems, Minneapolis, Minnesota, USA). IL-1β, IL-2, IL-6, TNF-α, MMP-2, and MMP-3 were measured using Luminex Multi-Analyte Profiling System (Luminex, Austin Texas, USA, Bio-Rad) an instrument that measures multiple analytes simultaneously in one sample [17, 18].

Statistical Analysis

The data are reported as the mean ± SE. The data between the two groups were compared using a 2-tailed unpaired Student's *t* test. Single linear univariate correlations (Pearson's correlation cofficients) were performed to evaluate the relationship between AGEs, sRAGE, or esRAGE, and cytokines and MMPs, between AGEs, AGEs/sRAGE, or AGEs/esRAGE, and cytokines, and between cytokines and MMPs. A p value of less than 0.05 was considered significant.

Results

Serum AGEs, sRAGE, and esRAGE

The serum levels of AGEs, sRAGE, and esRAGE in control subjects and in patients with TAA are summarized in Figure 1.

The serum levels of sRAGE were 1.43-fold lower (997.5 ± 84.06 vs. 1425.0±106.63 pg/ml), while levels

Table 1. Demographics and clinical characteristics of the patients with aortic aneurysms.

Parameter	Value (%)
Number of patients	20
Age (years) ± SD	53.85 ± 13.76
Male	15 (75%)
Female	5 (25%)
Race:	
African American	1 (5%)
Hispanic	1 (5%)
White	18 (90%)
Risk factors/comorbidities:	
Smokers (or history of smoking)	6 (30%)
Body mass index	34.29±0.25
Obesity	11 (55%)
Diabetes	3 (15%)
Hypertension	13 (65%)
Coronary artery disease	3 (15%)
Marfan syndrome	1 (5%)
Previous cardiac surgery	3 (15%)
Previous aortic surgery	1 (5%)
Positive family history	5 (25%)
Mean size of ascending aorta, cm	5.07±0.56

SD = standard deviation

of AGEs were 6.3 folds higher (20.06±3.69 vs. 2.93± 0.97 µg/ml) in patients with TAA compared to controls. The values for esRAGE were not significantly different from each other (0.372±0.0334 vs. 0.326±0.0391 ng/ml: controls vs. patient.)

AGEs/sRAGE, AGEs/esRAGE, MMP-2 and MMP-3

The ratio of AGEs/sRAGE, AGEs/esRAGE, and serum levels of MMP-2 and -3 for control subjects and patients with TAA are summarized in Figure 2. The ratio of AGEs/sRAGE and AGEs/esRAGE were 10.4- and 8.18-fold higher, respectively, in patients with TAA compared to controls. The values for MMP-2 and -3 were 29.60% and 142.1% higher in patients with TAA compared to controls, respectively.

Cytokines

The serum levels of IL-Iβ, IL-2, IL-6, and TNF-α in patients with TAA and control subjects are summarized in Figure 3. The values of IL-Iβ, IL-2, IL-6, and TNF-α in control subjects were 0.587±0.10, 0.402±0.066, 1.375±0.363, and 8.09±0.810 pg/ml, respectively. The levels of IL-Iβ, IL-2, IL-6, and TNF-α in patients with AA were, respectively, 3.49-, 22.38-, 10.65-, and 3.35-fold higher than controls, but the values were significant only for IL-2 and IL-6.

Correlation of sRAGE with IL-Iβ, IL-2, IL-6, and TNF-α

Correlation data from 20 patients with TAA and 11 control subjects are summarized in Figure 4. The levels of serum sRAGE are negatively correlated with IL-Iβ, IL-2, and I-6 but were significant only with IL-6. There was no correlation between sRAGE only and TNF-α.

Correlation of AGEs with Cytokines

The correlation between AGEs and cytokines is shown in Figure 5. The serum levels of AGEs are positively correlated with only IL-Iβ and IL-6. Although there was a tendency for negative correlation of AGEs with IL-2 and TNF-α, the correlation was not significant.

Correlation of AGEs/sRAGE with Cytokines

The results are summarized in Figure 6 and Table 2. There was a positive correlation between AGEs/ sRAGE, and IL-Iβ, IL-2, IL-6, and TNF-α but the correlation was significant only between AGEs/sRAGE and IL-2.

Correlation of esRAGE with Cytokines

There was a tendency for a positive correlation of serum esRAGE with serum IL-Iβ, IL-2, IL-6 and TNF-α, but the correlation was significant only between esRAGE and TNF-α (Table 2).

Correlation of AGEs/esRAGE with Cytokines

There was a tendency for a positive correlation between AGEs/esRAGE, and IL-Iβ and TNF-α, but the correlation was significant only between AGEs/ esRAGE and IL-2 and IL-6 (Table 2).
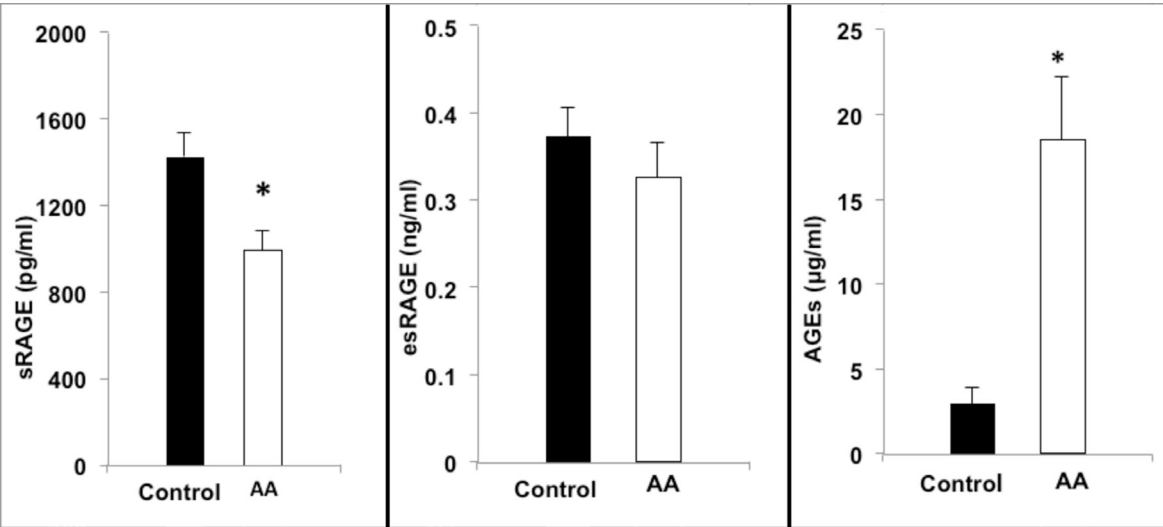
Figure 1. The serum levels of sRAGE, esRAGE, and AGEs in control subjects and in patients with ascending thoracic aortic aneurysms (AA). Results are expressed as the mean ± SE. AGEs = advanced glycation end products; sRAGE = soluble receptor for AGEs; esRAGE = endosecretory soluble receptor for AGEs. *P < 0.05; control vs. patients with AA.
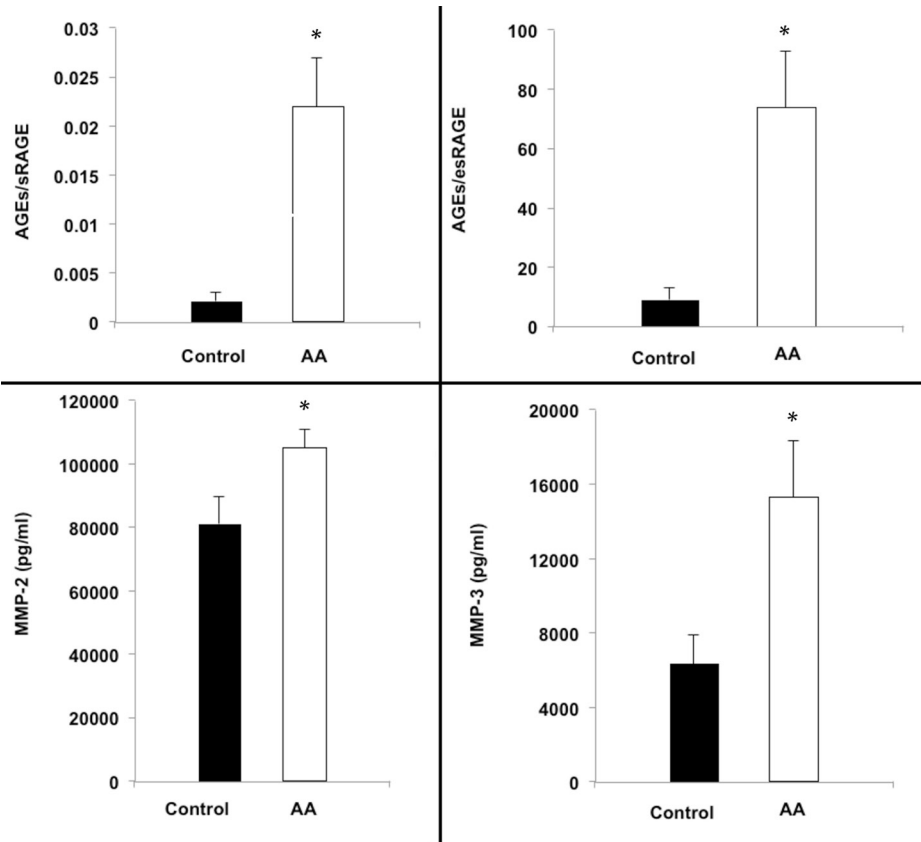

Figure 2. Ratios of AGEs/sRAGE, AGEs/esRAGE, and serum levels of MMP-2 and -3 in control subjects and patients with ascending thoracic aortic aneurysms (AA). Results are expressed as the mean ± SE. AGEs = advanced glycation end products; sRAGE = soluble receptors for AGEs; esRAGE = endosecretory soluble receptors for AGEs; MMP = matrix metalloproteinase. *P < 0.05, Control vs. patients with AA.
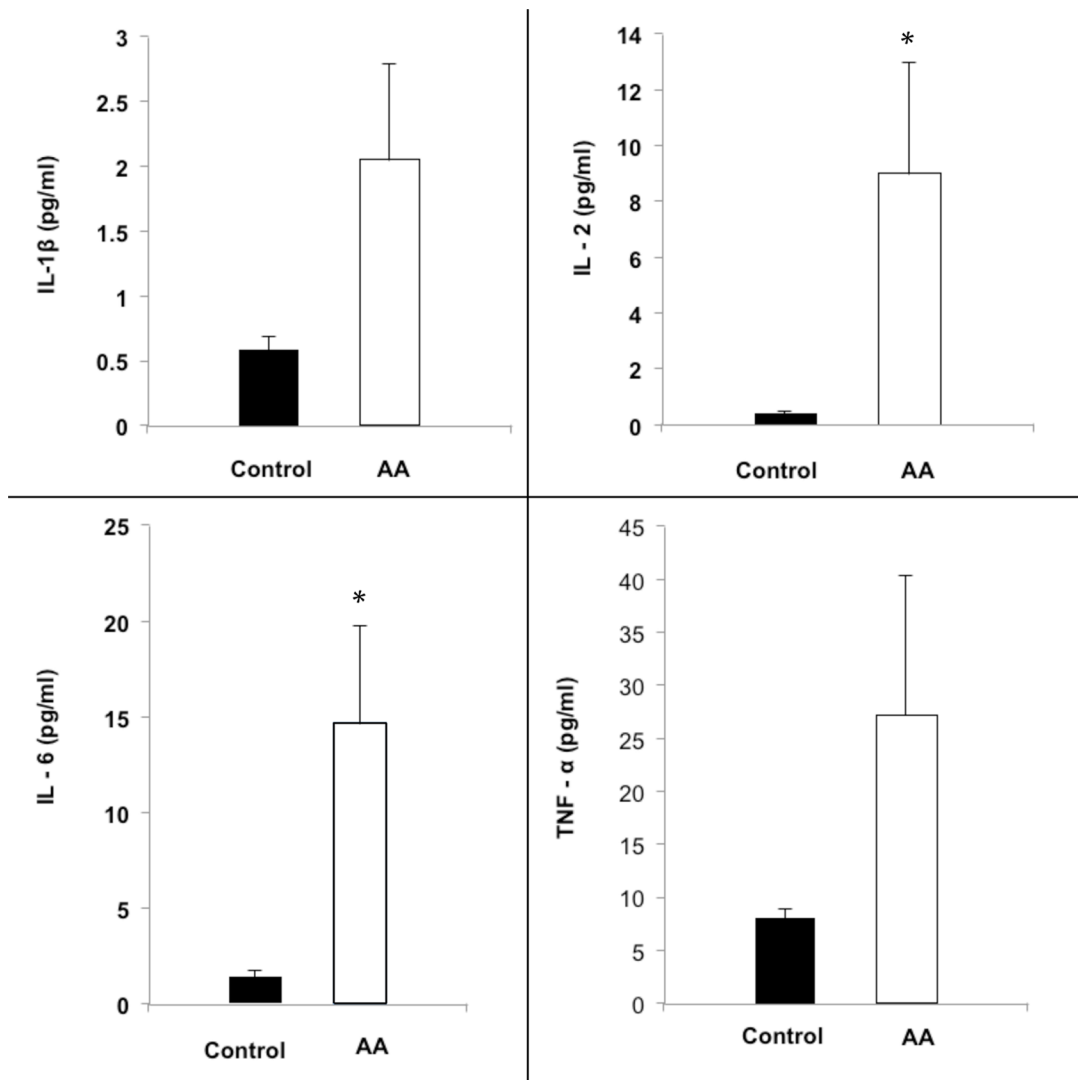
Figure 3. Serum levels of IL-Iβ, IL-2, IL-6, and TNF-α in control subjects and patients with TAA. Results are expressed as the mean ± SE. IL = interleukin; TNF-α = tumor necrosis factor-alpha. *P<0.05; control vs. patients with AA.

Correlation of MMP-2 or MMP-3 with sRAGE, esRAGE, AGEs, AGEs/sRAGE, and AGEs/esRAGE

The results are summarized in Figure 6 and Table 2. There was a tendency for a positive correlation between sRAGE and MMP-2, and a negative correlation between sRAGE and MMP-3 (Figure 6). There was a positive correlation of esRAGE with MMP-2 and -3 but not significant (Table 2). There was a weak positive correlation of AGEs with MMP-2 and negative correlation with MMP-3 (Table 2) and weak correlation of AGEs/sRAGE with MMP-2 and -3 (Figure 6), and between AGEs/esRAGE and MMP-2 and -3 (Table 2).

Correlation of Cytokines with MMP-2 and MMP-3

The correlation between cytokines and MMP-2 and -3 are summarized in Figure 7 and Table 2. There was a positive correlation between MMP-2 and IL-Iβ (significant), IL-2, IL-6, and TNF-α, and between MMP-3 and IL-Iβ (significant), IL-2, and TNF-α.

Discussion

The data show that serum levels of sRAGE are lower, and the levels of AGEs, AGEs/sRAGE, and AGEs/esRAGE are higher, in patients with TAA compared to
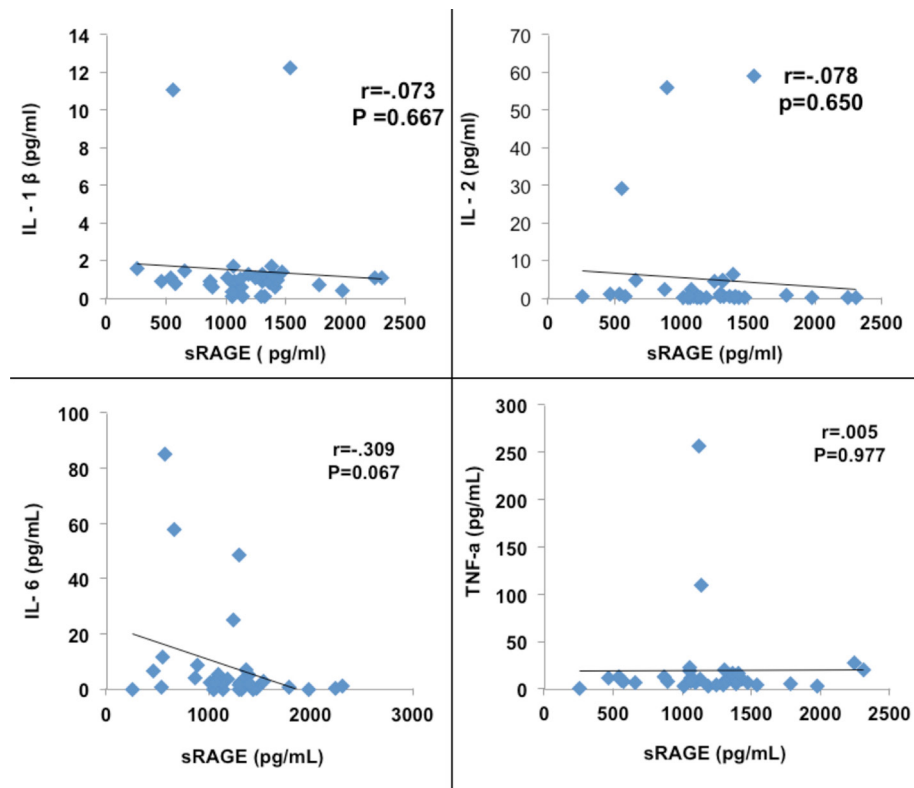
Figure 4. Correlation of sRAGE with IL-Iβ, IL-2, IL-6, and TNF-α. IL = interleukin; TNF-α = tumor necrosis factor-alpha; sRAGE = soluble receptor for advanced glycation end products.

control subjects. These are new findings. The levels of MMP-2, MMP-3, IL-Iβ, IL-2, IL-6, and TNF-α are higher in patients with TAA than in control subjects. Other investigators [19, 20] have also reported that the serum levels of IL-β, IL-2, IL-6, and TNF-α are elevated in patients with abdominal aortic aneurysm [19–21]. In the present study, although the levels of TNF-α were higher in patients with TAA compared to controls, they were not significantly different from each other. The elevated serum levels of MMP-2 and MMP-3 also have been reported in patients with abdominal aortic aneurysm and TAA [22–25].

In the present study there was a tendency for an inverse correlation between sRAGE and IL-β, IL-6, and IL-2. There was no significant inverse correlation between esRAGE and IL-Iβ, IL-2, and IL-6 except with TNF-α, which had a positive correlation with esRAGE. This positive correlation was not expected and the reason for this is not clear. There was a positive correlation between AGEs and cytokines (IL-Iβ and IL-6) and between

AGEs/sRAGE and IL-β or IL-6, but not with IL-2 or TNF-α. We had expected that there would be significant positive correlation between AGEs or AGEs/sRAGE and cytokines. It is possible that IL-Iβ and IL-6 are more frequently associated with the pathogenesis of TAA than are IL-2 and TNF-α.

There was a significant positive correlation of AGEs/esRAGE with IL-2 and IL-6 but not with TNF-α and IL-Iβ. Again it is not known why there was no positive correlation between AGEs/esRAGE and IL-Iβ and between AGES/esRAGE and TNF-α. May be that the sample size was too small to evoke all correlations that may eventually prove positive.

It was expected that low sRAGE and esRAGE would have negative correlation with MMP-2 and MMP-3, and AGEs/sRAGE would have positive correlation with MMP-2 and -3. However, it was found that only sRAGE have negative correlations with MMP-2 and AGEs/sRAGE have weak positive correlations with MMP-2 and -3.
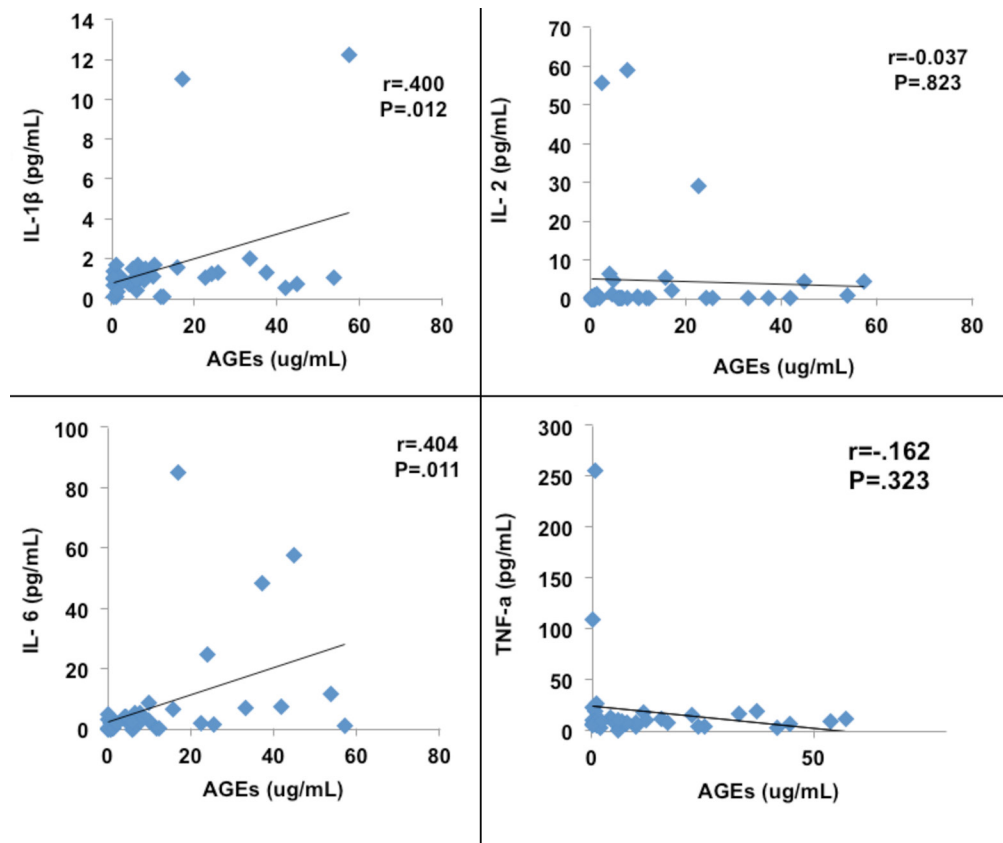
Figure 5. Correlation of AGEs with IL-Iβ, IL-2, IL-6, and TNF-α. AGEs = advanced glycation end products; IL = interleukin, TNF-α = tumor necrosis factor-alpha.
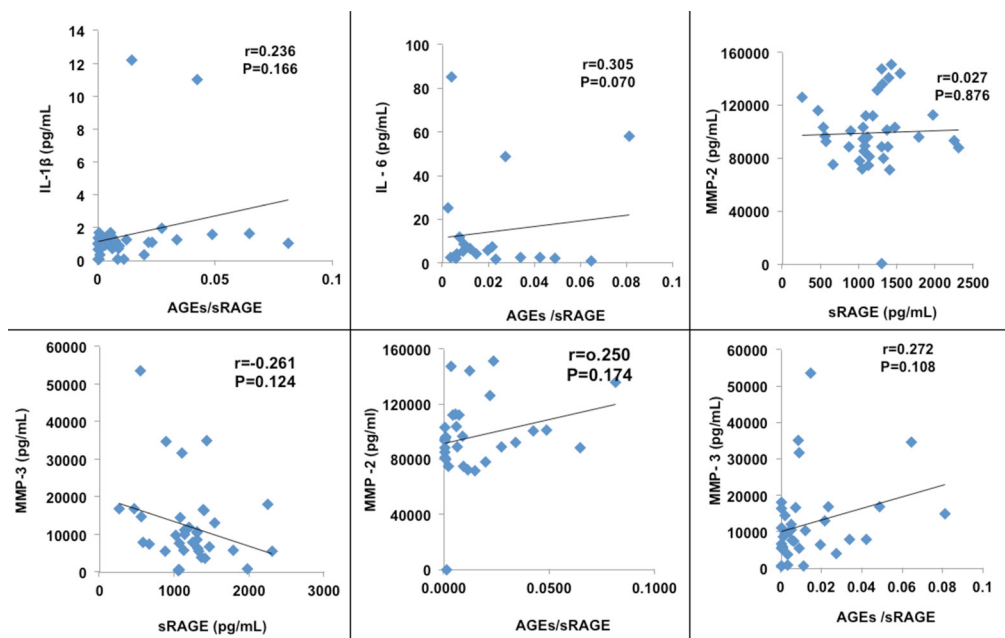

Figure 6. Correlation of AGEs/sRAGE with IL-Iβ, IL-6, MMP-2, and MMP-3, and of sRAGE with MMP-2 and -3. IL = interleukin; AGEs = advanced glycation end products; sRAGE = soluble receptor for AGEs; MMP = matrix metalloproteinase.

Table 2. Correlation coefficients (n=31).

Parameter	Pearson correlation	P value
IL-1β vs. esRAGE	0.223	0.227
IL-2 vs. esRAGE	0.291	0.113
IL-6 vs. esRAGE	0.124	0.507
TNF- α vs. esRAGE	0.380	0.035
MMP-2 vs. esRAGE	0.081	0.665
MMP-3 vs. esRAGE	0.007	0.969
IL-2 vs. AGEs/sRAGE	0.568	0.001
TNF-α vs. AGEs/sRAGE	0.277	0.131
IL-1β vs. AGEs / esRAGE	0.315	0.084
IL-2 vs. AGEs /esRAGE	0.371	0.040
IL-6 vs. AGEs / esRAGE	0.495	0.005
TNF-α vs. AGEs / esRAGE	0.159	0.392
MMP-2 vs. AGEs / esRAGE	0.152	0.415
MMP-3 vs. AGEs / esRAGE	0.015	0.934
MMP-2 vs. TNF-α	0.293	0.110
MMP-3 vs. TNF-α	0.017	0.927
MMP-2 vs. AGEs	0.176	0.343
MMP-3 vs. AGEs	-0.042	0.824

IL = interleukin; TNF-α = tumor necrosis factor-alpha; MMP = matrix metalloproteinase; AGEs = advanced glycation end products; esRAGE = endosecretory soluble receptors for AGEs.

Our hypothesis was that there would be positive correlation between IL-B, IL-2, or IL-6 and MMP-2 and -3. There was significant positive correlation of IL-Iβ with MMP-2 and -3, and a weak positive correlation of IL-2 with MMPs and IL-6 with MMP-2.

The data in general show that low levels of sRAGE and high levels of AGEs/sRAGE and AGEs/esRAGE are associated with an increase in the serum levels of cytokines and MMPs. Also, there is an inverse correlation between sRAGE and cytokines or MMP-3, and a positive correlation between AGE/sRAGE or AGE/esRAGE, and cytokines and MMPs. The serum levels of cytokines are positively correlated with MMPs.

In conclusion, the data suggest that low levels of sRAGE, and high levels of AGEs/sRAGE and AGEs/esRAGE, increase the levels of cytokines that in turn increase the levels of MMPs resulting in the formation of TAAs. The data suggest also that the AGE-RAGE axis may be involved in the pathogenesis of TAAs and that low levels of sRAGE and high levels of AGEs, AGES/sRAGE, and AGEs/esRAGE may be new risk factors for TAAs. This new mechanism of development of TAAs may open new avenues for the prevention, regression, and slowing of TAA progression. These findings may also help for
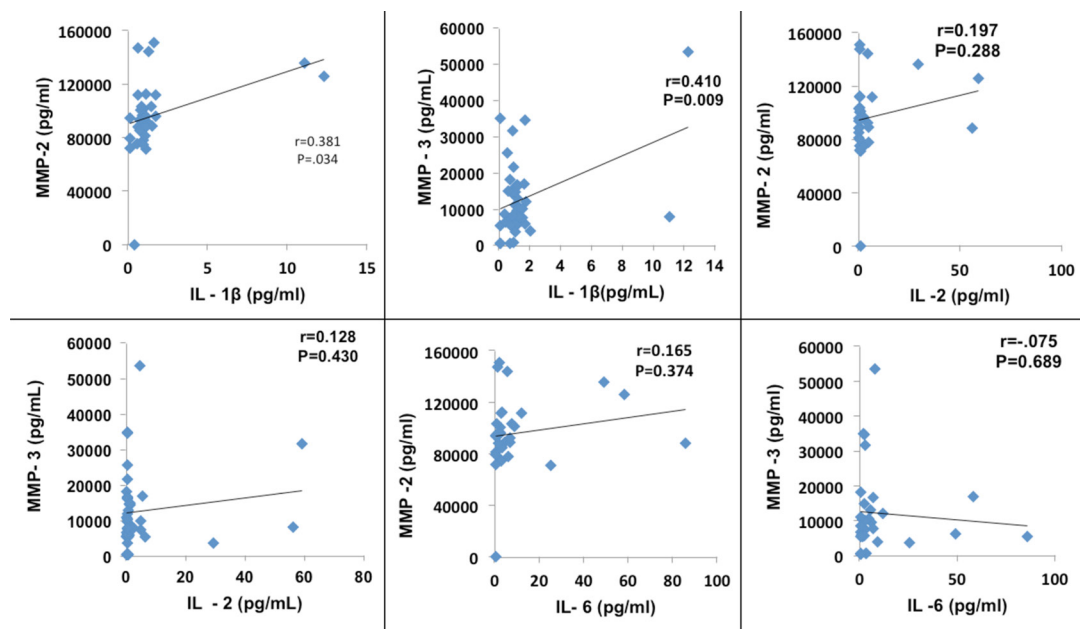
Figure 7. Correlation of IL-Iβ, IL-2, and IL-6 with MMP-2 and -3. IL = interleukin; MMP = matrix metalloproteinase.

screening of population for early detection of TAAs via biomarkers chosen from the pathophysiology herein demonstrated.

Acknowledgments

Conflict of Interest

The authors have no conflicts of interest relevant to this publication.

Comment on this Article or Ask a Question

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