



Serum levels of soluble receptor for advanced glycation endproducts (sRAGE) are higher in ulcerative colitis and correlate with disease activity

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Abstract

Interaction of the receptor for advanced glycation endproducts (RAGE) with its ligands results in expression of inflammatory mediators, activation of NF- κ B, and induction of oxidative stress, all of which have been implicated in the pathogenesis of inflammatory bowel diseases (IBD). Soluble receptor for advanced glycation endproducts (sRAGE) has recently emerged as a reliable biomarker of inflammation in numerous RAGE-mediated disorders.

Objective: To assess sRAGE levels in adult patients with IBD.

Method: Serum was collected from adult patients with Crohn's disease (CD, 56 patients), ulcerative colitis (UC, 60 patients), and healthy controls (HC, 113 subjects). Levels of sRAGE were determined by enzyme-linked immunosorbent assay.

Results: Serum sRAGE levels were elevated in IBD compared to HC and were higher in UC patients compared to CD and HC. Levels of sRAGE were significantly higher in the serum of UC patients with active disease compared to patients with inactive disease, but no association with the Montreal Classification was evident. Serum sRAGE was lower in CD patients with biological therapies.

Conclusions: These findings suggest that serum levels of sRAGE are altered in patients with intestinal inflammation and may reflect distinct immunoinflammatory pathogenesis of UC and CD.

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1. Introduction

The receptor for advanced glycation endproducts (RAGE) is a cell-surface member of the immunoglobulin superfamily and a multiligand receptor interacting with a number of different

proinflammatory ligands, including advanced glycation end-products and S100 calcium-binding proteins.^{1,2} The interaction of cell-surface RAGE with its proinflammatory ligands results in an increased oxidative stress and activation of NF- κ B, which in turn leads to increased expression of proinflammatory genes and further generation of oxygen radicals.³ RAGE has secretory isoforms referred to as soluble RAGE (sRAGE), which comprise the extracellular ligand-binding domain but are lacking the cytosolic and transmembrane domains.⁴ Soluble RAGE has the same ligand binding specificity of cell-bound RAGE and may serve as a decoy abrogating cellular activation.⁵ In human serum, soluble RAGE can exist as a number of different isoforms, which include endogenous secretory RAGE (esRAGE) – formed through alternative splicing – and an isoform generated by ectodomain shedding of cell-surface RAGE.⁵ The measurement of soluble RAGE isoforms in human serum is facilitated by two distinct ELISA systems. The most commonly used total sRAGE ELISA measures the total pool of soluble RAGE using antibodies that recognize both the spliced and cleaved forms of sRAGE.⁵ Another assay specifically measures esRAGE in serum by another ELISA due to its unique C-terminus sequence.⁶ Importantly, there is evidence to suggest that circulating levels of total sRAGE may serve as a reliable biomarker of inflammation.⁷

The pathogenesis of inflammatory bowel diseases (IBD) remains largely unknown, but preliminary data have suggested a potential role of RAGE and its ligands in the onset and perpetuation of chronic intestinal inflammation. Foell et al. have shown that the RAGE ligand S100A12 is strongly upregulated during chronic active IBD.⁸ Andrassy and co-workers have reported that inflamed gut biopsy tissue demonstrates a significant upregulation of RAGE and increased NF-kappaB activation.⁹ Zen et al. subsequently confirmed that RAGE expression is increased in colon tissue samples from patients with IBD and suggested that RAGE mediates neutrophil adhesion to, and subsequent migration across, intestinal epithelial monolayers.¹⁰ Cirillo and colleagues have suggested that the binding of S100B to RAGE is involved in the increased nitric oxide production occurring in the mucosa of patients with ulcerative colitis.¹¹ Intriguingly, Leach and colleagues have reported that several RAGE ligands are increased in children with IBD, while no significant elevation of sRAGE levels was found.¹²

Currently there is a lack of knowledge regarding the role of circulating sRAGE in adult patients with IBD. The aim of the present study was therefore to report on measurement of serum sRAGE in an adult population of patients with IBD. We also investigated the associations of sRAGE with disease activity and treatment.

2. Materials and methods

2.1. Study participants

Three groups of adult subjects were enrolled in the present study: subjects with Crohn's disease (CD, $n=56$), ulcerative colitis (UC, $n=60$), and healthy controls (HC, $n=113$). All subjects with IBD were recruited from the Department of Gastroenterology, Marmara University, School of Medicine, Istanbul, Turkey. The diagnosis of Crohn's disease was

established by a combination of clinical evaluation with endoscopic, histological, radiological, and/or biochemical investigations. The diagnosis of UC was established by a combination of medical history, clinical evaluation, and typical endoscopic and histological findings. Infectious causes of intestinal diseases were excluded in all patients. Patients with indeterminate colitis were not included in this study. All subjects in the control group were blood donors judged to be in good health. Subjects with disorders known to alter sRAGE levels such as coronary artery disease, stroke, diabetes, dementia, nonalcoholic fatty liver disease, renal failure, and arthritis were excluded.⁴ A written informed consent was obtained from all participants. The study protocol was approved by the Institutional Review Board of the Marmara University School of Medicine.

2.2. Clinical assessment

Types of Crohn's disease were classified as inflammatory, stenosing, fistulizing, and stenosing plus fistulizing. Location of Crohn's disease was classified as 0, ileum; 1, colon; 2, ileocolonic; 3, gastroduodenal; and 4, other locations. The Montreal Classification¹³ was applied to assess the extent of ulcerative colitis with E1: ulcerative proctitis; E2: left-sided UC (distal UC); and L3: extensive UC (pancolitis). Remission was defined as an Crohn's Disease Activity Index (CDAI)¹⁴ < 150 in CD, and a Activity Index (AI)¹⁵ < 150 for UC.

2.3. Biochemical assays

Blood samples were collected in the fasting state and centrifuged at 2500 g for 10 min. Serum samples were stored at -80°C until analyzed. Serum sRAGE levels were determined using a commercially available ELISA kit (Catalog Number RD191116200R, BioVendor, Brno, Czech Republic) according to the manufacturer's protocol. Measurements were performed in duplicate and the results were averaged. Personnel responsible for performing assays had no knowledge of clinical data. The intra-assay and interassay coefficients of variation were <6 and <8%, respectively.

2.4. Data analysis

Sample size was calculated with the GraphPad StateMate 2.0 software (GraphPad, Inc., San Diego, CA, USA) by the estimated power for two-sample comparison of means of log-transformed sRAGE values. On the basis of previous results in control groups⁴ we expected a 15% elevation of the primary endpoint (serum sRAGE), needing a sample size of 30 subjects for controls and 55 in the IBD group, with an 80% power to detect a significant difference (with a two-sided type 1 error of 5%). We therefore recruited in excess of this figure to be fully confident in our data. Continuous data were checked for normal distribution using the Kolmogorov–Smirnov statistics. Normally distributed data are shown as means \pm SDs. Skewed variables are expressed as median and interquartile ranges. Differences in the levels of sRAGE among the study groups were assessed by Kruskal–Wallis analysis followed by Tukey's multiple-comparison post-hoc test. Correlations among the study variables were tested by the Spearman's correlation coefficient. We used a

multivariable logistic regression analysis, adjusted for age, gender, diabetes, serum CRP, erythrocyte sedimentation rate, and serum albumin, to determine the significant factors indicating presence of UC. All calculations were generated with the use of SPSS 17.0 (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism 4.0 (GraphPad, Inc., San Diego, CA, USA). The level of significance was set as a two-tailed $P < 0.05$.

3. Results

3.1. Levels of sRAGE in IBD

Table 1 depicts the general characteristics of the three study groups. In the 56 patients with CD, the type of disease was inflammatory in 18 subjects, stenosing in 11 patients; fistulizing in 17 patients, and stenosing and fistulizing in 10 patients. Localization of CD was as follows: ileum in 15 patients; colon in 11 patients; ileo-colonic in 30 patients. No patient had gastroduodenal or other locations of CD. Three patients had extraintestinal manifestations of CD. According to the Montreal Classification for patients with UC,¹² there were 4 patients with ulcerative proctitis, 26 with left sided UC (distal UC) and 30 with extensive UC (pancolitis).

Serum sRAGE was elevated in the entire group of 116 patients with IBD (CD plus UC) compared to HC [median (interquartile range): 677 (534–810) versus 580 (470–770) pg/mL, respectively, $P < 0.05$]. When calculations were performed for CD, UC and controls separately (Table 1), levels of sRAGE were significantly higher in UC patients compared to CD and control subjects. No significant difference was found between CD patients and healthy controls (Fig. 1). Serum levels of sRAGE were not associated with CRP, serum albumin, and erythrocyte sedimentation rate. Multivariable logistic regression analysis adjusted for age, gender, serum CRP, erythrocyte sedimentation rate,

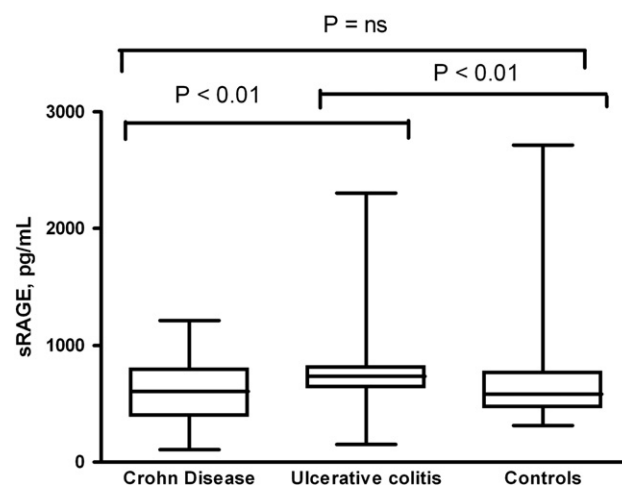


Figure 1 Box plots showing serum values of sRAGE levels in patients with Crohn's disease (n=56), ulcerative colitis (n=60) and healthy controls (n=113). The bottom and top of the box are the 25th percentile and the 75th percentile.

and serum albumin, identified sRAGE as independently associated with the presence of UC (odds ratio=1.004, 95% confidence interval=1.000–1.008, $P < 0.05$).

3.2. Correlation of sRAGE to disease activity and clinical characteristics

Levels of sRAGE were significantly higher in the serum of UC patients with active disease [n=19, 823 (710–910) pg/mL] compared to patients with inactive disease [n=41, 710 (670–808) pg/mL, $P < 0.05$]. Serum sRAGE was not associated with the Montreal Classification for patients with UC. In addition, sRAGE was not related to any relevant classifying aspects of CD, including type and localization of the disease (data not shown).

Table 1 General characteristics of patients with Crohn's Disease (CD), ulcerative colitis (UC), and healthy controls (HC).

	CD	UC	HC	P-value
Number	56	60	113	
Males/Females	30/26	29/31	50/63	
Age (years)	41 ± 12	44 ± 16	43 ± 15	
Active/inactive	5/51	19/41		
Disease duration (years)	5 (2–9)	4 (2–7)		
Serum CRP (mg/dL)	3.9 (2.9–8.5)	3.2 (2.9–6.2)		
Erythrocyte sedimentation rate (mm/h)	20 (11–36)	13 (9–23)		
Serum albumin (g/dL)	4.3 ± 0.7	4.4 ± 0.6		
Medications				
5-ASA	20	24		
Sulfasalazin	4	13		
Corticosteroids	4	5		
Infliximab/adalimumab	16	1		
Azathioprine	8	13		
Antibiotics	1	0		
No treatment	3	4		
sRAGE (pg/mL)	610 (400–800)	740 (640–820)	580 (470–770)	<0.001 ¹ , <0.05 ²

¹ Significant difference between the three study groups by Kruskal-Wallis test.

² Between UC and HC; CRP: C-reactive protein, 5-ASA: 5-aminosalicylic acid.

3.3. Treatment and sRAGE levels

Serum sRAGE was lower in CD patients with biological therapies (infliximab, adalimumab) [n=16, 490 (340–730) pg/mL] compared to patients without this treatment [n=40, 710 (550–920) pg/mL, $P<0.05$]. In contrast, no association with treatment was seen in patients with ulcerative colitis. Of note, patients with CD not treated with biological therapies had still significantly lower levels of sRAGE [n=40, 710 (550–920) pg/mL] than the subgroup of UC patients with active disease [n=19, 823 (710–910) pg/mL, $P<0.05$].

4. Discussion

To the best of our knowledge, this is the first study reporting on serum sRAGE levels in adult patients with IBD. There are two principal findings in the present investigation. First, serum sRAGE levels were elevated in IBD compared to HC and higher in UC patients with active disease compared to CD and HC. Second, serum sRAGE was significantly reduced in CD patients with biological therapies. This indicates disease- and clinical-related differences in serum sRAGE levels in IBD.

RAGE is a ubiquitously expressed receptor for non-enzymatically glycosylated adducts of endogenous proteins, lipids and nucleic acids capable to bind to numerous proinflammatory molecules including S100 proteins.¹⁶ Previous data have shown that RAGE and its ligands are highly hyperexpressed in the intestinal mucosa of patients with IBD.^{8–11} These data suggest a role for RAGE and its ligands in the augmentation of intestinal injury and has provided us a impetus for further research into the role of sRAGE as a biomarker of IBD. An important finding of our study was the elevation of serum sRAGE levels with active UC but not in CD. Because RAGE and its ligands affect the function of immune cells involved in the pathogenesis of IBD,^{10,11} the differences in circulating levels of sRAGE may be related to the distinct clinical features of CD and UC. The association of elevated sRAGE levels with active UC may reflect increased expression of cell-bound RAGE in the intestinal mucosa of these patients. Of note, total sRAGE in serum is thought to mainly reflect RAGE forms that are proteolytically cleaved from cellular surface by matrix metalloproteinases and are then shed into the bloodstream.^{17,18} Since we did not perform cell-surface RAGE expression analyses in intestinal biopsies from our subjects, we cannot specify the origin of the elevated serum sRAGE levels in active UC. It is plausible, however, that it may be the result of increased shedding of RAGE forms from cell-surface RAGE through metalloproteases that are highly expressed in active UC.¹⁹ Alternatively, sRAGE may be up-regulated as a countermeasure to prevent tissue/cell damage owing to its decoy effect toward proinflammatory RAGE ligands.²⁰ Our findings that sRAGE levels were significantly higher in the serum of UC patients with active disease compared to patients with inactive disease support this possibility. We hypothesize that a more active inflammatory status of our patients may be reflected by increased sRAGE levels. Noteworthy is that Leach et al. have previously failed to detect a significant elevation of sRAGE in a pediatric population of patients with IBD.¹² However, only four children with UC were enrolled in this study, and the lack of significant differences in sRAGE levels might be due to a lack of power.

Serum sRAGE was reduced in CD patients receiving biological therapies. Intriguingly, Foell et al. have previously shown that levels of S100A12, a ligand for RAGE, decreased rapidly after treatment with infliximab.⁸ It is conceivable that modulation of RAGE expression might be an additional effect of anti-TNF- α treatment in the setting of IBD. Of note, blockade of the S100A12/RAGE interaction in mice has been shown to result in a suppression of chronic colonic inflammation accompanied by reduced TNF- α in serum.²¹

Although this is the first demonstration of an association between high levels of sRAGE and active UC in adult individuals, there are several limitations to our study. Our results are mainly limited by the small number of subjects enrolled and by cross-sectional design that allowed only demonstration of a modest association between sRAGE and active UC, therefore definitive conclusions cannot be drawn. At present, we do not know whether total sRAGE levels could be mechanistically related to intestinal inflammation by reflecting tissue RAGE expression. However, previous studies have already reported an upregulation of mucosal RAGE in IBD.^{8–11} Second, the enzyme-linked immunosorbent assay used in this study quantifies concentrations of total sRAGE in serum. This assay cannot differentiate between native secretory RAGE isoforms and soluble RAGE that results from the cleavage of the cell-surface receptor by metalloproteinases.⁴ Third, our sample included subjects of Turkish nationality, so that results cannot be extrapolated to populations with different ethnic background. Importantly, a recent multiethnic study has shown that significant differences exist in terms of sRAGE levels in distinct ethnic groups, possibly as a result of genetic influences.²² Fourth, the sRAGE levels we observed were lower than those previously published (for instance¹²) due to a different immunosensitivity of the ELISA system used and consequently are not comparable with the other studies. Fifth, this study lacks the evaluation of RAGE expression in the intestinal tissue that could provide a more complete picture of the complex interaction between tissue RAGE and sRAGE in IBD. Finally, despite the differences in serum levels of sRAGE between UC and CD, a crucial prerequisite for the clinical use of biomarkers is elucidation of analytical features, standardization of analytical methods, assessment of performance characteristics, and demonstration of cost-effectiveness. A new biomarker for distinguishing UC from CD will be of clinical value only if it is reproducibly obtained in a standardized fashion, it is easy to interpret by clinicians. Further studies are needed to shed more light on this important issue.

In conclusion, we demonstrate for the first time that sRAGE is elevated in the serum of active UC patients. These findings further support a role of RAGE in intestinal inflammation that may be possibly associated with the distinct immunopathogenesis of UC and CD. Further progress in understanding the role of the RAGE in IBD is warranted possibly to devise novel therapeutic strategies.

Conflict of interest

YY carried out the studies and data analyses and drafted the manuscript. OY, FE and OA carried out the samples analyses. FE participated in the design of the study and performed the statistical analysis. HOH conceived the study, and participated in its design and coordination and helped to draft

the manuscript. All authors read and approved the final manuscript.

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