Alexandria Journal of Medicine

ISSN: 2090-5068 (Print) 2090-5076 (Online) Journal homepage: https://www.tandfonline.com/loi/tajm20

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To cite this article: Anna Nashaat Abou-Raya, Maher Abdel Nabi Kamel, Eman Abdel Ghani Sayed & Ahmed Abdel Hamid El-Sharkawy (2016) The plasma level of soluble receptor for advanced glycation end products in systemic lupus erythematosus patients and its relation to disease activity, Alexandria Journal of Medicine, 52:2, 151-157, DOI: 10.1016/j.ajme.2015.06.005

To link to this article: https://doi.org/10.1016/j.ajme.2015.06.005

Published online: 17 May 2019.

Alexandria Journal of Medicine (2016) **52**, 151–157

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Alexandria Journal of Medicine

http://www.elsevier.com/locate/ajme

ORIGINAL ARTICLE

The plasma level of soluble receptor for advanced glycation end products in systemic lupus erythematosus patients and its relation to disease activity

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Received 13 January 2015; accepted 23 June 2015
Available online 23 July 2015

KEYWORDS

Advanced glycation end products;
Systemic lupus erythematosus;
High mobility group box-1

Abstract *Introduction:* In recent years, the role of high mobility group box-(HMGB-1) protein and its receptors has received increasing attention. It has been documented that HMGB-1 is associated with disease activity in systemic lupus erythematosus (SLE). HMGB-1 supports the inflammatory clearance of apoptotic cells and remnants. It binds to molecules released from apoptotic cells such as nucleosomes and DNA thereby, increasing the immunogenicity of macrophages through receptors for advanced glycation end products (sRAGE).
Aim of the work: Was to measure the plasma level of sRAGE in SLE patients and to correlate it with the clinical and laboratory parameters of disease activity.
Patients and methods: The study was composed of 35 SLE patients; 31 females and 4 males (Group I) and 20 age and gender matched healthy subjects as a control (Group II). All patients fulfilling the American College of Rheumatology (ACR) classification criteria for the diagnosis of SLE. Active disease was identified using SLE disease activity index (SLE-DAI).
Demographic data, cutaneous manifestations, arthritis, vasculitis, myositis, renal, and hematological disorders were recorded. In addition; complete blood picture, blood urea, serum

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Peer review under responsibility of Alexandria University Faculty of Medicine.

http://dx.doi.org/10.1016/j.ajme.2015.06.005

creatinine, 24 h urine proteins, creatinine clearance, protein/creatinine ratio, C3, C4, Anti-nuclear antibody, Anti-double stranded DNA were conducted for all patients and controls.

Results: The mean value of plasma level of (sRAGE) in SLE patients was significantly higher in SLE patients than in the normal healthy controls ($P < 0.001$). There was a statistically significant positive correlation between sRAGE and SLE-DAI ($P < 0.001$).

Conclusion: The plasma level of sRAGE is considered as a potential biomarker for disease activity in SLE, severity and prognosis.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by the involvement of multiple organ systems. Its etiology is largely unknown; however, it has been proposed that genetic and environmental factors contribute to breaking tolerance, resulting in the production of a variety of antibodies directed at self-components.[1]

These autoantibodies form immune complexes can be deposited in many tissues, particularly the skin and kidneys.[2,3] Currently, research is being conducted to determine what patho-physiological mechanisms are involved in this entire process.

Receptor for advanced glycation end products (RAGE) is a multi-ligand member of the immunoglobulin super-family. It is expressed by most types of immune cells, including macrophages, neutrophils, and T cells and interacts with several classes of ligands.[4] Currently, the known RAGE ligands include the high mobility group box-1 (HMGB1) protein, advanced glycation end products (AGEs), and members of the S100/calgranulin family.[5]

One of the proinflammatory mediators is the HMGB1. It was originally recognized as a DNAbinding protein but has recently been identified as a damage-associated molecular pattern (DAMP) molecule.[6,7] This nuclear protein participates in chromatin architecture and transcriptional regulation,[8] but once released, it induces an inflammatory response.[9,10] Extracellular HMGB1 binds to cell surface receptors, including RAGE, toll-like receptors 2 and 4, and others. Studies have shown that interaction between HMGB1 and RAGE results in the production of type -1 interferon, which plays a key role in the pathogenesis of SLE.[11,12] In addition, tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) are produced upon HMGB-1 activation of macrophages.[13]

It has also been hypothesized that these cytokines also affect some body organs as well as disease flareups.[14,15] In addition, it has also been postulated that RAGE involvement in all pathophysiological processes is reliant on HMGB1.[16] Some studies have reported a relationship between the high serum level of HMGB1 and flare-ups of lupus disease activity.[17,18] All of these observations support the notion that the HMBG1–RAGE pathway plays a part in the pathogenesis of SLE.

Another class of ligands is the AGEs. They result from a process in which non-enzymatic glycosylation attaches to circulating compounds such as lipids, proteins, or nucleic acids. This process occurs under the effect of oxidative stress (OS) and hyperglycemia.[19] Accumulations of AGEs have been found in certain diseases, including diabetes mellitus (DM) and Alzheimer's. Since RAGE induction is under the effect of AGEs, the RAGE-AGEs pathway is thought to be incriminated in the pathogenesis of these diseases.[20,21]

Receptor for advanced glycation end products is a receptor for a family of about 20 related calcium binding proteins that are only expressed in vertebrates. These include the S100s, which are proteins that alter several intracellular functions.[22]

In addition, many types of body cells release S100s during inflammation; therefore, they can be used as a measure of disease activity.[23,24]

Soluble RAGE (sRAGE), a truncated form of the receptor, has the same structure but lacks the cytosolic and transmembrane domains. Two general mechanisms are usually responsible for the generation of soluble receptors. These are either derived from the alternative splicing of messenger ribonucleic acid (mRNA) or the cleaved products of the membrane-bound form of metalloproteinase.[25,26] Both sRAGE and complete RAGE have the same ligand-binding specificity. Furthermore, sRAGE may act as a trap for pro-inflammatory ligands such as HMGB1 and inhibit their interaction with the RAGE cell surface.[27,28]

2. Aim of the work

Was to measure the plasma level of sRAGE in SLE patients and to correlate it with the clinical and laboratory parameters of disease activity.

3. Patients and methods

The study was composed of 35 patients with SLE 31 females and 4 males (Group I) and 20 age and gender matched healthy subjects (Group II).

All of the patients conformed to the American College of Rheumatology (ACR) classification criteria for the diagnosis of SLE.[29] Active disease was identified using the systemic lupus erythematosus Disease Activity Index (SLE-DAI).[30]

Demographic and clinical data, including cutaneous manifestations, arthritis, vasculitis, myositis, renal disorders were recorded. In addition; complete blood picture,[31] blood urea nitrogen, serum creatinine, 24 h urine proteins, protein/creatinine ratio, creatinine clearance,[32] C_3, C_4,[33] anti-nuclear antibody, (ANA),[34], and anti-double stranded DNA (Anti-ds DNA)[35] were conducted for all patients and controls.

Plasma concentrations of sRAGE levels were measured using enzyme Linked Immunosorbant Assay (ELISA).

Table 1 Comparison between the two studied groups according to demographic data.

	Cases (n = 35)		Control (n = 20)		Test of sig	P
	No.	%	No.	%		
Sex						
Male	4	11.4	2	10.0	χ^2 = 0.027	^{FE}P = 1.000
Female	31	88.6	18	90.0		
Age						
Min–max	18.0–52.0		18.0–42.0		t = 0.277	P = 0.783
Mean ± SD	33.60 ± 11.53		33.60 ± 9.52			
Median	31.0		30.50			
Disease duration (years)						
Min–max	3.0–10.0		–		–	–
Mean ± SD	6.43 ± 2.08		–			
Median	7.0		–			

P: p value for comparing between cases and control groups.
χ^2 = value of Chi square.
FE: Fisher Exact test.
t = Student-test.

Table 2 The clinical description and frequency of organ affection among the studied group.

	Cases (n = 35)	
	No	%
Arthritis	27	77.1
Fever & malaise	14	40.0
Myositis	13	37.1
Pleurisy	12	34.3
Oral ulcers	9	25.7
Photosensitivity	6	17.1
Malar rash	5	14.3
Psychosis	4	11.4
Pericarditis	4	11.4
Hair fall	2	5.7
Seizures	1	2.9
Min–max	1.0–35.0	
Mean ± SD	13.83 ± 7.61	
Median	15.0	

Patients with other connective tissue diseases were excluded from the study, and also patients with diabetes, endocrinal diseases, and HCV positive patients.

Written consent was taken from all patients included in the study.

3.1. Statistical analysis of the data

Data were processed and analyzed using a computer based program (SPSS software version 10). The results were expressed as mean ± standard deviation (SD), and comparisons between the SLE patients and the controls were analyzed by the student's t-test, one way analysis of variance (ANOVA), and spearman's rank correlation coefficient. A two tailed P-value of <0.05 was considered to be statistically significant.

4. Results

Table 1: describes the distribution of the studied cases; 31 cases (88.6%) were females and 4 cases (11.4%) were males. Their age ranged from 18.0 to 52.0 with a mean of 33.60 ± 11.53. The disease duration ranged from 3.0 to 10.0 with a mean of 6.43 ± 2.08. Table 2: describes the distribution and frequency of organ involvement in SLE cases; 27 cases (77.1%) presented with arthritis, 14 cases (40.0%) presented with fever and malaise, 13 cases (37.1%) cases presented with myositis, 12 cases (34.3%) presented with pleurisy, 9 cases (25.7%) presented with oral ulcer, 6 cases (17.1%) presented with photosensitivity, 5 cases (14.3%) presented with malar rash, 4 cases (11.4%) presented with psychosis, 4 cases (11.4%) presented with pleurisy, 2 cases (5.7%) presented with hair fall, and 1 case (2.9%) presented with seizures. Table 3: describes the laboratory markers of the studied SLE cases; Hb % ranged from 8.0 to 10.50 with a mean of 9.41 ± 1.69, WBCs ranged from 2.40 to 3.51 with a mean of 3.37 ± 2.18, platelet count ranged from 80.0 to 130.0 with a mean of 117.46 ± 64.93, blood urea ranged from 60.0 to 458.0 with a mean of 64.70 ± 7.66, serum creatinine ranged from 2.32 to 8.20 with a mean of 2.50 ± 2.84, creatinine clearance ranged from 95.0 to 130.0 with a mean of 104.30 ± 8.94 and 24-h urine creatinine ranged from 40.0 to 4000.0 with a mean of 1637.43 ± 1587.86. Table 4: shows the immunological markers among the studied SLE cases; ANA ranged from 160.0 to 450.0 with a mean of 249.83 ± 230.57, anti-ds-DNA ranged from 250.0 to 600.0 with a mean of 355.17 ± 257.15, C_3 level ranged from 95.0 to 160.0 with a mean of 147.70 ± 17.44, and C_4 level ranged from 84 to 160 with a mean of 64.57 ± 48.03. Table 5: shows the distribution of the studied SLE cases according to SLE-DAI Scores; 4 cases (11.4%) were with mild SLE-DAI, 5 cases (14.3%) were with moderate SLE-DAI, and 26 cases (74.3%) were with severe SLE-DAI. Table 6: shows comparison between the two studied groups according to sRAGE; in SLE sRAGE ranged from 1124.0 to 1413.0 with a mean of 1253.54 ± 66.74, while in the control group ranged from 824.0 to 1118.0 with a mean of 975 ± 25.01. Table 7:

Table 3 Comparison between the two studied groups according to laboratory markers.

	Cases (n = 35)	Control (n = 20)	t	P
HB (gm/dl)				
Min–max	8.0–13.50	11.0–15.0	4.867*	< 0.001*
Mean ± SD	10.41 ± 1.69	12.23 ± 1.07		
Median	9.50	12.0		
WBCs ($\times 10^3$ cell/mm^3)				
Min–max	2.40–9.51	4.0–9.51	0.788	0.434
Mean ± SD	5.37 ± 2.18	5.83 ± 1.87		
Median	4.70	4.95		
Platelets ($\times 10^3$ cell mm^3)				
Min–max	90.0–330.0	150.0–330.0	0.406	0.686
Mean ± SD	217.46 ± 64.93	224.45 ± 54.65		
Median	220.0	220.0		
Serum Creatinine (mg/dl)				
Min–max	0.32–8.20	0.32–1.30	1.695	0.090
Mean ± SD	2.50 ± 2.84	0.89 ± 0.24		
Median	0.90	0.85		
Blood urea (mg/dl)				
Min–max	20.0–458.0	20.0–178.0	2.090*	0.037*
Mean ± SD	34.70 ± 7.66	67.08 ± 2.84		
Median	37.0	40.0		
Creatinine (ml/min) clearance				
Min–max	95.0 ± 130.0	7.2 ± 110.0	5.106*	< 0.001*
Mean ± SD	104.30 ± 8.94	56.17 ± 36.23		
Median	102.0	54.30		
24 h urine protein: (mg/24 h)				
Min–max	40.0–4000.0	10.0–150.0	5.114*	< 0.001*
Mean ± SD	1637.43 ± 1587.86	61.75 ± 45.86		
Median	1000.0	40.0		

t = Student t-test for comparing between cases and control groups.
P: p value for comparing between cases and control groups.
* Statistically significant at $p \leqslant 0.05$.

Table 4 Comparison between the two studied groups according to immunological markers. (ANA, Anti-ds DNA, C3, C4).

	Cases (n = 35)	Control (n = 20)	Z	P
ANA (N < 1/40 IU/L)				
Min–max	160.0–450.0	5.0–30.0	3.923*	< 0.001*
Mean ± SD	249.83 ± 230.57	15.66 ± 5.76		
Median	100.0	15.0		
Anti-ds DNA (N ⩽ 30 IU/L)				
Min–max	250.0–600.0	5.0–20.0	4.853*	< 0.001*
Mean ± SD	355.17 ± 257.15	22.50 ± 12.30		
Median	380.0	20.0		
C3(N 75–135 mg/dl)				
Min–max	85.0–160.0	75.0–120.0	6.127*	< 0.001*
Mean ± SD	147.70 ± 17.44	53.49 ± 14.55		
Median	150.0	50.0		
C4 (N12–75 mg/dl)				
Min–max	84–160	5.0–40.0	5.140*	< 0.001*
Mean ± SD	64.57 ± 48.03	18.05 ± 8.15		
Median	61.50	16.0		

Z: Z for Mann Whitney test for comparing between cases and control groups.
* Statistically significant at $p \leqslant 0.05$.

Table 5 Distribution of the studied SLE cases according to SLE-DAI scores.

SLE-DAI	No.	%
Inactive	0	0.0
Mild	4	11.4
Moderate	5	14.3
Severe	26	73.3
Min–max	5.0–35.0	
Mean ± SD	17.83 ± 7.61	
Median	18.0	

shows correlation between sRAGE and frequency of organ involvement among the studied group; there was a positive correlation between sRAGE and the presence of arthritis ($r = 0.608^{*}$, $P < 0.001$), fever and malaise ($r = 0.602^{*}$, $P < 0.001$), pericarditis ($r = 0.624^{*}$, $P < 0.001$), hair fall ($r = 0.904^{*}$, $P < 0.001$), and seizures ($r = 0.624^{*}$, $P < 0.001$). Table 8: shows correlation between sRAGE and C_3, C_4, ANA Anti-ds DNA, SLE-DAI, 24-h urine proteins, and protein/creatinine ratio; there was a positive correlation between sRAGE and SLE-DAI ($r = 0.947^{*}$, $P < 0.001$), 24-h urine proteins ($r = 0.545^{*}$, $P < 0.001$), and protein/creatinine ratio ($r = 0.575^{*}$, $P < 0.001$).

5. Discussion

Systemic lupus erythematosus (SLE) is a chronic, multifaceted inflammatory disease that can affect every organ system of the body. SLE is variable in its manifestations and follows a relapsing and remitting course.[36] HMGB-1 seems to be an important molecule in the pathophysiology of SLE by supporting the inflammatory clearance of apoptotic cells by binding to molecules released from apoptotic cells such as nucleosomes and DNA thereby increasing the immunogenicity of macrophages through receptors for advanced glycation end products (RAGE) to initiate cell signaling, this uptake accompanied by inflammation.[37,38]

The advanced glycation end products may bind to cell surface receptors to initiate cell signaling. The advanced glycation end products receptor includes R1, wR2, R3, scavenger receptors SCR-2, CD-36, and RAGE.[39]

As plasma sRAGE levels increased in patients with SLE, this suggests that sRAGE may play a different role in the initial and progressive stages of the disease. However, the use of anti-lupus therapy could be involved in the triggering of a compensating mechanism that alters sRAGE production and/or regulation.

In our study, the plasma level of sRAGE in SLE studied group of patients, ranged from 1124.0 to 1412.0 with a mean of 1253 ± 66.74 while, in the control group sRAGE ranged from 824.0 to 1118.0 with a mean of 975.30 ± 25.01. There was a statistically significant difference between SLE patients and controls as regards sRAGE ($P < 0.001$). Also, there was a significant positive correlation between sRAGE and SLE-DAI ($P < 0.001$).

In a study carried by Martens et al.,[40] receptor for advanced glycation end products (RAGE) was associated with systemic lupus erythematosus disease activity and severity.

Another study carried by Ma et al.,[41] the role of high mobility group box-1 (HMGB1) protein and its receptors in autoimmune diseases and documented that HMGB1 is associated with disease activity in patients with SLE. Also, plasma sRAGE in patients receiving long-period treatment was significantly increased compared to those with short-period treatment and comparable with those in healthy control.

Table 7 Correlation between sRAGE and frequency of organ involvement among the studied group.

	sRAGE	
	r	*P*
Arthritis	0.608*	< 0.001
Fever & malaise	0.602*	< 0.001
Myositis	0.904*	< 0.001
Pleurisy	0.605	0.005
Oral ulcers	0.456	0.039
Photosensitivity	0.570	0.009
Malar rash	−0.532	0.015
Psychosis	−0.533	0.016
Pericarditis	0.624*	< 0.001
Hair fall	−0.609	0.004
Seizures	0.614*	< 0.001

Table 8 Correlation between sRAGE and C3, C4, ANA Anti-ds DNA, SLE-DAI, 24-h urine proteins, and protein/creatinine ratio.

	sRAGE	
	r	*P*
C3	−0.035	0.843
C4	−0.132	0.451
ANA	0.231	0.383
Anti-ds-DNA	0.241	0.163
SLE-DAI	0.947*	< 0.001*
24 h-urine proteins	0.545*	< 0.001*
Protein/creatinine ratio	0.575 *	< 0.001*

r: Pearson coefficient.
* Statistically significant at $p \leqslant 0.05$.

Table 6 Comparison between the two studied groups according to sRAGE.

	Cases ($n = 35$)	Control ($n = 20$)	*t*	*P*
sRAGE (ng/dl)				
Min–max	1124.0–1412.0	824.0–1118.0	6.215*	< 0.001*
Mean ± SD	1253.54 ± 66.74	975.30 ± 25.01		
Median	1250.0	1171.0		

In another study done by Chavakis et al.,[42] sRAGE is a multiligand receptor that propagates cellular dysfunction in several inflammatory disorders. RAGE is expressed at low levels in normal tissues, but becomes upregulated at sites where its ligand accumulate. RAGE may play a dual role in the inflammatory responses. sRAGE interaction occurs on leukocytes or endothelial cells with its ligands results in cellular activation involving the transcription factor NF-kappa B: on the other hand, sRAGE on endothelial cells may function as an adhesive receptor that directly interacts with leukocyte SS2-integrins, thereby directly being involved in inflammatory cell recruitment.

In another study by Millard et al.[28] the formation of advanced glycation end products (AGEs) is a result of the non-enzymatic reaction between sugars and free amino groups of proteins. AGEs, through interaction with their specific receptor for AGEs (RAGE), result in activation of pro-inflammatory states and involved in numerous pathologic conditions. The soluble form of RAGE is able to act as a decoy to avoid interaction of RAGE with its pro-inflammatory ligands (AGES, HMGB1, S100 proteins).

The results of the study emphasize the possible involvement of the sRAGE pathway in the immunopathogenesis of SLE, which could lead it to have a potential therapeutic role.

6. Recommendations

HMGB1 is a nuclear DNA-binding protein that residues inside the nucleus detected in renal biopsy of patients with lupus nephritis. Further studies are promptly needed in order to evaluate the importance in determining urinary HMGB-1 and its value as a biomarker in lupus nephritis patients with renal involvement.

HMGB1 can be released to the extra-cellular space under specific conditions. Whereas HMGB1 is actively released from lipopolysaccharide (LPS), TNF, IL-1 activated monocytes and macrophages, its release also occurs passively during late phase of apoptosis as well as during necrosis.

Urinary biomarkers might be more relevant than serum biomarkers and could detect both systemic and local inflammation, they directly reflect renal pathology and possibly, a therapeutic target.

Conflict of interest

We have no conflict of interest to declare.

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