

# Serum salusin- $\alpha$ levels in systemic lupus erythematosus and systemic sclerosis

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## Abstract

**Objective:** Systemic lupus erythematosus (SLE) and systemic sclerosis (SSc), chronic inflammatory diseases, demonstrate an increased incidence of cardiovascular manifestations and subclinical atherosclerotic disease. Salusin- $\alpha$  is a novel bioactive peptide that suppresses the formation of macrophage foam cells, and its serum level is significantly lower in patients with angiographically proven coronary artery disease. The aims of the study were to assess serum salusin- $\alpha$  level and its potential association with the predictors of atherosclerosis in SLE and SSc.

**Material and Methods:** The study included 20 SLE and 22 SSc patients and 23 healthy controls (HC). All of the participants were female. Tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-6 and salusin- $\alpha$  levels, *homeostasis model assessment for insulin resistance* (HOMA-IR) index and common carotid intima-media thickness (IMT) were determined.

**Results:** Salusin- $\alpha$  levels were lower and the IMTs were higher in the SLE and SSc groups than in the HC group. The salusin- $\alpha$  level was correlated with neither the disease activity scores nor cytokine levels and IMT in the SLE and SSc groups, although it was correlated with triglyceride level in the SLE group ( $r=-0.564$ ,  $p=0.012$ ), and with HOMA-IR index in the HC group ( $r=0.485$ ,  $p=0.019$ ).

**Conclusion:** The present preliminary study may support the idea that SSc leads to subclinical atherosclerosis, as in SLE. Moreover, it can be concluded that the decreased salusin- $\alpha$  levels in SLE and SSc may contribute to subclinical atherosclerosis. However, further studies with larger sample size are needed to demonstrate this contribution in SLE and SSc.

**Key words:** Systemic lupus erythematosus, systemic sclerosis, atherosclerosis, salusin- $\alpha$

## Introduction

Salusin- $\alpha$  and salusin- $\beta$  are multifunctional endogenous bioactive peptides, comprised of 28 and 20 amino acids, respectively, which were newly identified by Shichiri et al. (1). Salusin peptides are synthesised from preprosalusin, an alternatively spliced product of the torsion dystonia-related gene (TOR2A) (1). Salusins are expressed and synthesised within human tissues including vessels, the central nervous system, and kidneys (1), as well as from human monocytes and macrophages (2). Furthermore, the presence of salusins in human plasma and urine has also been demonstrated (3, 4).

Salusins mainly affect the cardiovascular system (5-8). Salusin- $\beta$  is the most hypotensive peptide: its infusion rapidly and profoundly decreases blood pressure (BP) and heart rate (5). Moreover, it is demonstrated to cause cardiac dysfunction via a cholinergic mechanism in rats (5). Yu et al. (6) have reported that salusins may have important roles in myocardial growth and hypertrophy. Salusin- $\alpha$  and salusin- $\beta$  show inverse actions on atherosclerosis due to their opposite regulatory effects on *acyl-coenzyme A: cholesterol acyltransferases-1* (ACAT-1) (7). Both the formation of macrophage foam cells and the development of atherosclerosis are suppressed by salusin- $\alpha$  (7). Serum salusin- $\alpha$  levels are also reported to be significantly lower in patients with coronary artery disease, and in hypertensive patients in whom the salusin- $\alpha$  level is inversely associated with carotid atherosclerosis (7-9).

Systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) are chronic inflammatory diseases. Patients with SLE and SSc are at an increased risk of developing atherosclerosis even after adjustment for traditional risk factors of cardiovascular diseases (10-16). The cause of accelerated atherosclerosis in chronic inflammatory diseases is a still mystery (10-16). Therefore, the aim of the present study was to determine the serum salusin- $\alpha$  levels in the cohorts of patients with SLE and SSc, and to establish their possible effects on the *homeostasis model assessment for insulin resistance* (HOMA-IR) index and intima-media thickness (IMT) of the common carotid artery in these patients.

## Material and Methods

### Participants

Twenty female patients with SLE, 22 female patients with SSc, and 23 healthy female controls (HCs) were included in this study. Patients were recruited from those treated and followed-up in the Rheumatology



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Department of Firat University Hospital, Elazig, Turkey. HCs were selected from staff members employed in our institute. SLE was diagnosed according to the American College of Rheumatology (ACR) revised classification criteria for SLE (17). SSc was diagnosed according to the ACR preliminary classification criteria of SSc (18). The study protocol was approved by the institutional Ethics Committee, and all of the participants gave informed consent before enrolling in the study.

Detailed histories of all of the participants were obtained and systemic and rheumatologic examinations were performed. Corticosteroid usage was also recorded. For all of the participants, body weight (BW) and body height (BH) were measured to determine the body mass index (BMI), expressed as:  $BMI = BW \text{ (kg)} / BH \text{ (m)}^2$ . Patients with hypertension, diabetes mellitus, liver or kidney diseases and endocrine disorders, those receiving statins, smokers, and those with a history of atherosclerosis and/or familial dyslipidaemia were excluded from this study.

The SLE activity status was assessed using the SLE Disease Activity Index (SLEDAI) (19). SLEDAI is a global index including 24 weighted objective clinical and laboratory variables. Disease activity can range from 0 to 105, and active SLE was defined as a SLEDAI score of  $\geq 6$  (19). The disease damage and severity was determined by the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) damage index in the SLE group (20). In the SSc group, the disease activity was determined by the Valentini disease activity index (21), while the disease damage and severity were determined by Medsger disease severity scale (22).

#### Laboratory analysis

Overnight fasting blood samples were drawn from all the participants. Serum samples were stored at  $-20^\circ\text{C}$  until further analysis. Routine laboratory evaluation of complete blood cell count, fasting blood glucose (FBG), lipid profile, hepatic and renal function tests, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) were assessed in patients and controls using standard laboratory methods.

Serum insulin and C-peptide levels were assayed with method of *chemiluminescence assay* using appropriate commercial kits (Diagnostic Products Corporation, Los Angeles, CA, USA). Insulin resistance was detected by the HOMA-IR index using the following equation:  $HOMA-IR = [\text{fasting blood insulin } (\mu\text{U/mL}) \times \text{FBG (mmol/L)}] / 22.5$ .

Serum tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin (IL)-6 levels were determined using by enzyme-linked immunosorbent assay (ELISA) methods following the manufacturer's instruc-

tions (BioSource International, Inc. Camarillo, California, USA). Serum salusin- $\alpha$  levels were measured using an appropriate commercial kit (Phoenix Pharmaceuticals, Inc. Burlingame, CA, USA) by the radioimmunoassay (RIA) method.

#### Carotid intima-media thickness

The subjects were studied in the early afternoon under standardised conditions, in a quiet room at a comfortable temperature. All participants had fasted before testing and were asked to refrain from strenuous exercise or drinking alcohol or caffeine-containing beverages for 24 h before the study. Upon arrival at the investigation unit, the subjects were equipped with measurement devices and then rested supine for about 15-20 min, until heart rate and mean BP trends demonstrated that satisfactory baseline conditions had been achieved. Arterial BP and carotid artery values were measured during the last 5 min of the resting period. All study cases underwent carotid ultrasonography; all studies were performed by an experienced research sonographer using an identical protocol and were interpreted by a single cardiologist, who was blinded to the subjects' clinical and laboratory findings. The common carotid arteries were evaluated with high-resolution B-mode ultrasonography using an echotomographic system (Acuson Sequoia 512 machine; Acuson, Minnesota, USA) with a 7.5 MHz linear transducer. Patients were examined in the supine position, with the neck rotated  $45^\circ$  in the direction opposite that which was being examined. IMT was measured on the far wall at 5, 10, and 15 mm proximal to the carotid bifurcation, over both the right and left common carotid arteries. The IMT was defined as the distance from the leading edge of the first echogenic line to the leading edge of the second echogenic line. Reproducibility of the IMT measurement was deemed acceptable, as demonstrated by a coefficient of variation (CV) of 3% for the IMT. The mean IMT, defined as the mean of the six measurements (three for each side), was used for statistical analysis.

#### Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS 11.0, Chicago, IL, USA). Results were presented as mean  $\pm$  standard deviation (SD). The normal distribution of the variables was evaluated with the Kolmogorov-Smirnov test, and logarithmic transformations were performed to normalise data with skewed distribution (insulin, TNF- $\alpha$  and IL-6). Statistical differences among the groups were identified with one-way analysis of variance (ANOVA) followed by the Tukey's post hoc test. Chi-square test was used to compare the categorical variables. Correlation analysis was performed using the Pearson correlation coefficient. Analysis of covariance (ANCOVA) was also used to adjust variables for age and BMI. *P* values less than 0.05 were considered to be significant.

#### Results

The demographics and laboratory data of the SLE, SSc, and HC groups are summarised in Tables 1 and 2. The mean age was higher in the SSc group than in the SLE group ( $p < 0.05$ ), while the mean ages in the SLE and SSc groups were similar to those of the HC group ( $p > 0.05$  for both). The SLEDAI and SLICC/ACR scores were  $8.1 \pm 6.3$  and  $0.75 \pm 1.2$ , respectively, and the disease duration was  $4.0 \pm 4.1$  years, in the SLE group. The Valentini and Medsger scores were  $3.6 \pm 1.3$  and  $6.5 \pm 2.5$ , respectively, and the disease duration was  $4.7 \pm 7.1$  years in the SSc group. The ESR level was higher in the SLE group than in the HC group ( $p < 0.001$ ). The CRP levels were similar among all three groups. The levels of TNF- $\alpha$  and IL-6 were higher in the SLE ( $p < 0.001$  and  $p < 0.05$ , respectively) and SSc (for both  $p < 0.01$ ) groups than in the HC group. When compared with the HC group, the high-density lipoprotein cholesterol (HDL-C) level was lower in the SSc group ( $p < 0.01$ ). The FBG and insulin levels and HOMA-IR indexes were similar among all groups. However, the C-peptide levels were higher in the SLE and SSc groups ( $p < 0.001$  and  $p < 0.01$ , respectively) than in the HC group. Moreover, while 4.3% of

**Table 1.** Demographics and clinical characteristics of the SLE, SSc, and HC groups

	SLE (n=20)	SSc (n=22)	HC (n=23)	p
Age (years)	35.8 $\pm$ 10.9	44.4 $\pm$ 13.6 <sup>a</sup>	39.5 $\pm$ 9.2	0.054*
BMI (kg/m <sup>2</sup> )	23.9 $\pm$ 4.6	25.4 $\pm$ 4.4	26.4 $\pm$ 5.1	0.234*
Systolic BP (mmHg)	107 $\pm$ 13	110 $\pm$ 13	102 $\pm$ 15	0.175*
Diastolic BP (mmHg)	65 $\pm$ 8	70 $\pm$ 10 <sup>b</sup>	63 $\pm$ 9	0.020**
Disease duration (years)	4.0 $\pm$ 4.1	4.7 $\pm$ 7.1	-	0.328***
Corticosteroid usage (%)	95	36.4	-	<0.001**
Corticosteroid dose (mg/d) <sup>#</sup>	7.6 $\pm$ 9.3	5.0 $\pm$ 6.6	-	0.403***

Data were presented as mean  $\pm$  SD.

p values of \*ANOVA, \*\*Chi-square and \*\*\*Student's t tests.

The significance of difference detected by the Tukey's post hoc test when compared to the SLE group: <sup>a</sup> $p < 0.05$ ; when compared to the HC group: <sup>b</sup> $p < 0.05$ .

<sup>#</sup>equivalent prednisolone dosage of corticosteroids.

SLE: systemic lupus erythematosus; SSc: systemic sclerosis; HC: healthy controls; BMI: body mass index; BP: blood pressure

**Table 2.** Laboratory data for the SLE, SSc and HC groups

	SLE (n=20)	SSc (n=22)	HC (n=23)	P <sub>(ANOVA)</sub>
Total cholesterol (mg/dL)	179±69	173±35	206±37	0.063
LDL-C (mg/dL)	128±43	112±25	132±29	0.109
HDL-C (mg/dL)	46±12	41±13 <sup>b</sup>	55±13	0.002
Triglyceride (mg/dL)	154±81	131±53	122±42	0.217
FBG (mg/dL)	84±9	95±22	89±9	0.355
Insulin (IU/mL) <sup>†</sup>	10.5±6.9	7.9±4.3	7.3±3.2	0.099
C-peptide (ng/mL)	3.3±1.5 <sup>c</sup>	3.1±1.2 <sup>b</sup>	2.1±0.6	0.002
HOMA-IR	2.2±1.5	1.9±1.8	1.6±0.7	0.419
ESR (mm/h)	42.6±24.6 <sup>c</sup>	30.6±19.4	19.0±9.4	<0.001
CRP (mg/L)	7.6±8.5	11.5±15.9	5.6±1.4	0.332
TNF- $\alpha$ (pg/mL) <sup>†</sup>	19.5±15.2 <sup>c</sup>	13.4±5.3 <sup>b</sup>	8.7±4.8	<0.001
IL-6 (pg/mL) <sup>†</sup>	7.3±8.1 <sup>a</sup>	11.4±18.1 <sup>b</sup>	3.8±5.3	0.007
Salusin- $\alpha$ (pg/mL)	86.7±1.8 <sup>a</sup>	91.7±2.0	94.8±2.5	0.053
IMT (mm)	0.644±0.08 <sup>c</sup>	0.684±0.08 <sup>c</sup>	0.538±0.03	<0.001

SLE: systemic lupus erythematosus; SSc: systemic sclerosis; HC: healthy controls; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; FBG: fasting blood glucose; HOMA-IR: homeostasis model assessment for insulin resistance; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; TNF: tumour necrosis factor; IL: interleukin; IMT: intima-media thickness. Data were presented as mean±SD.

<sup>†</sup>Logarithmic transformations were applied to data with skewed distribution before statistical analysis.

The significances of differences when compared to the HC group: <sup>a</sup>p<0.05, <sup>b</sup>p<0.01, <sup>c</sup>p<0.001.

the control group and 13.6% of the SSc group (odds ratio [OR]: 2.1, 95% confidence interval [CI]: 0.3-36.2, p=0.273) had insulin resistance, it was relatively frequent in the SLE group (25% of SLE patients had insulin resistance, and OR: 3.5, 95% CI: 0.8-69.2, p=0.051).

The IMTs were higher in both patient groups than in the HC group (p<0.001 for both). After adjustment for age, the increase in IMTs remained significant (ANCOVA p<0.001 for both). In the SLE group, the IMT was correlated with the age (r=0.478, p=0.033) and total cholesterol (r=0.479, p=0.032). The IMT was correlated with the systolic BP (r=0.461, p=0.031), diastolic BP (r=0.433, p=0.039), triglyceride (r=0.694, p=0.001), and C-peptide (r=0.459, p=0.019) levels in the SSc group.

Salusin- $\alpha$  levels were lower in the SLE group than in the HC group (Table 2). After adjustment for age, it was significantly lower in the both SLE and SSc groups than in the HC group (ANCOVA p<0.001 for both). However, it was correlated with neither the disease activity scores nor cytokine levels and IMTs in the SLE and SSc groups. The salusin- $\alpha$  level was correlated positively with the fasting insulin level and HOMA-IR index in the HC group (r=0.488, p=0.018 and r=0.485, p=0.019, respectively). It was also correlated with the triglyceride and HDL-C levels in the SLE group (r=-0.564, p=0.012 and r=0.518, p=0.023, respectively).

Overall, 95% of SLE patients were receiving corticosteroids; therefore, the potential effect of corticosteroid usage on the study parameters could not be analysed. In the SSc group, 36.4% of patients were receiving corticoste-

roids. The serum fasting insulin, C-peptide, acute phase reactants, cytokines and salusin levels and HOMA-IR index were similar in the SSc patients receiving corticosteroids and those who were not (p>0.05 for all). However, the mean value of IMT was significantly higher in the SSc patients receiving corticosteroids than in those not receiving them (0.73±0.09 vs. 0.66±0.05 mm, p=0.027), while total cholesterol (149.6±26.1 vs. 186.1±33.4 mg/dL, p=0.017) and low-density lipoprotein cholesterol (95.7±24.3 vs. 119.5±21.9 mg/dL, p=0.036) levels were significantly lower in the former subgroup.

## Discussion

In the present study, serum salusin- $\alpha$  level and its potential relation on carotid atherosclerosis in the SLE and SSc were determined. The serum salusin- $\alpha$  levels were lower in the SLE and SSc groups which have higher IMTs, in the present study.

In an epidemiologic study (10), SLE patients have been demonstrated to have a 50-fold increased risk of developing myocardial infarction than the general population. The relative risk for coronary heart diseases has still been reported to be 7- to 17-fold higher, even after adjusting for traditional risk factors in SLE (12). Therefore, these reports have suggested that patients with SLE possess additional risks in addition to the traditional risk factors for the development of accelerated atherosclerosis. In contrast to SLE, it is controversial whether SSc leads to accelerated and premature atherosclerosis. Hettema et al. (23) reported that SSc is not associated with an increased prevalence of early signs of atherosclerosis.

Similarly, a retrospective study showed that the prevalence of coronary artery disease was not frequent in SSc (24). On the other hand, it has been demonstrated that patients with SSc have increased incidence of atherosclerotic diseases (13-16). Moreover, two recently published meta-analyses of atherosclerosis in SSc have found that SSc patients have a higher prevalence of coronary atherosclerosis, peripheral vascular disease, and cerebrovascular calcification as well as an increased carotid IMT, which is a surrogate marker of atherosclerosis (25, 26). Our study also confirmed an increased prevalence of subclinical atherosclerosis in SSc in addition to SLE.

Salusin- $\alpha$  and salusin- $\beta$  are considered to be biosynthesised from preprosalusin, an alternatively spliced product of TOR2A (1). The expressions of preprosalusin and TOR2A in human monoblastic leukaemia cell lines THP1 and U937 have been demonstrated (27). Moreover, salusin- $\alpha$  and salusin- $\beta$  have been reported to be expressed by inflammatory cells (2, 7). It has been also demonstrated that the release of salusin- $\beta$  from human monocytes/macrophages is stimulated by lipopolysaccharide and TNF- $\alpha$  (2). Previously, our group has reported higher salusin- $\alpha$  levels in chronic inflammatory diseases including rheumatoid arthritis (RA) and Behçet's disease (BD) (28). However, in the present study, salusin- $\alpha$  levels were decreased in female SLE and SSc patients.

Salusin- $\alpha$  and salusin- $\beta$  are biosynthesised from the same precursor, preprosalusin (1); however, the regulatory mechanisms for the biosynthesis of salusins are not fully known. Sato et al. (2) have reported that TNF- $\alpha$  enhances salusin- $\beta$  expression from U937 cells, but not from THP-1 cells, while IL-1 $\beta$  has no effect on the expression of salusin- $\beta$  from both cell lines. Therefore, it may be concluded that different cytokines may lead to different effects on the expression of salusin, while the responses of different cells to the same cytokines may also be different. It is well known that CD4+ helper T cells are subdivided into different subsets, including Th1 and Th2, according to the cytokine secretion pattern. RA and BD are Th1-mediated diseases, while SLE and SSc are Th2-mediated diseases. In contrast to the increased salusin- $\alpha$  levels in RA and BD (28), its decreased levels in SLE and SSc can suggest that salusin- $\alpha$  may be involved in the inflammatory response of Th1-mediated diseases.

Salusins, a new class of multifunctional bioactive peptides, have been reported to regulate haemodynamics and atherogenesis as well as the immune system (1, 7). Salusin- $\alpha$  has anti-atherogenic effects, while salusin- $\beta$  has pro-atherogenic effects (7). In human-monocyte-derived macrophages, salusin- $\alpha$  has been

reported to inhibit cholesterol ester accumulation and ACAT-1 expression, both of which are associated with the transformation of macrophages to foam cells (7). However, salusin- $\beta$  has induced ACAT-1 protein expression, while higher doses of salusin- $\alpha$  are required to inhibit the stimulatory effect of salusin- $\beta$  on ACAT-1 protein expression (7). In addition, the level of salusin- $\alpha$  is decreased in coronary artery disease and inversely correlated with IMT (7-9), although a subsequent clinical study has documented that carotid artery plaque score is not negatively correlated with serum salusin- $\alpha$  level in patients with renal failure (29). In our study, whereas any direct correlation between the serum salusin- $\alpha$  level and IMT was not observed, the decreased serum salusin- $\alpha$  levels accompanied increased IMTs in the SLE and SSc groups. Thus, it may be speculated that decreased salusin- $\alpha$  level may contribute to the accelerated atherogenesis in SLE and SSc.

The present study has some limitations. The sample size could have been too small to reach definitive judgments. The level or expression of salusin- $\beta$  could be evaluated. The analysis of salusin- $\beta$  is technically more difficult than that of salusin- $\alpha$ , since salusin- $\beta$  adheres to tubes and rapidly disappear (30). The control group could have included participants with hypertension or atherosclerotic diseases. Carotid IMT is well-validated surrogate marker of atherosclerosis, and has been documented to predict future cardiovascular events in healthy individuals (31). However, the presence of carotid artery plaques, which may be a stronger predictor of atherosclerotic diseases (32), was not evaluated in our study.

The serum salusin- $\alpha$  levels were lower in the female SLE and SSc patients with higher IMTs, whereas there was no correlation between salusin- $\alpha$  levels and the IMTs in each group in the present study. Therefore, it can be speculated that the increased IMTs in the SLE and SSc groups may be associated with the decreased salusin- $\alpha$  levels. However, the sample size of the present study was too small to draw definite conclusions. Thus, further studies with a larger sample size are needed to understand the regulation of salusin- $\alpha$  and determine its relation with predictors of atherosclerosis in SLE and SSc.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Institutional Ethics Committee.

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author contributions:** Concept - S.S.K.; Design - S.S.K.; Supervision - A.I.; Resource - S.S.K.; Materials - S.S.K.; Data Collection&/or Processing - M.Ö.; Analysis&/or Interpretation - B.Ü., M.N.D.; Literature Search - B.I.; Writing - S.S.K.; Critical Reviews - S.S.K., A. I.

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