Association between serum total antioxidant status and flow-mediated dilation in patients with systemic lupus erythematosus: an observational study

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Abstract

Objective: Endothelial dysfunction (ED) is a condition that involves increased oxidative stress and decreased total antioxidant status (TAS) levels. Systemic lupus erythematosus (SLE) is also associated with ED. We aimed to determine the association between serum TAS and ED as assessed by flow-mediated dilation (FMD) in patients with SLE.

Methods: Thirty-four patients with stable SLE who were not undergoing any treatment and 39 healthy volunteers without any overt cardiovascular disease were included in this cross-sectional study. Doppler ultrasound was used to measure FMD to assess ED in the study groups. Serum TAS levels were measured using a TAS kit. High-sensitivity C-reactive protein (hs-CRP) and anticardiolipin antibody (aCLA) levels were also measured to assess the inflammatory state. The SLE group further was divided into 2 groups according to presence or absence of aCLA. SLE disease activity was assessed using the SLE disease activity index (SLEDAI). Regression analysis was used to define independent predictors.

Results: The mean TAS levels were significantly lower in patients with SLE than in controls (1.60±0.11 versus 1.73±0.15 mmol/L, p<0.001). hs-CRP levels were significantly higher in patients with SLE than in controls (8.2±6.0 vs. 2.9±4.0 mg/L; p<0.001), particularly in SLE patients with positive aCLA when compared with SLE patients with negative aCLA (13.8±4.3 vs. 5.6±4.8 mg/L, p<0.001). The FMD percent was significantly lower in patients with SLE than in controls (8.1±4.9 vs. 10.6±4.7, p=0.04). There was a significant positive correlation between FMD and TAS in the SLE group (r=0.448, p=0.008) and the control group (r=0.367, p=0.03) and a significant negative correlation between FMD and serum hs-CRP (r=-0.368, p=0.04) in only the SLE group. In multiple linear regression analysis, TAS, hs-CRP, and SLEDAI were independently correlated with FMD (β=0.50, p=0.003; β=-0.33, p=0.03; and β=-0.36, p=0.03; respectively).

Conclusion: Patients with SLE who have no overt cardiovascular disease are at increased risk for ED and this may be associated with underlying inflammation and impairment of TAS. *(Anatol J Cardiol 2015; 15: 913-8)*

Keywords: endothelial dysfunction, flow-mediated dilation, high-sensitivity C-reactive protein, systemic lupus erythematosus, total antioxidant status

Introduction

Systemic lupus erythematosus (SLE) is a chronic, multisystemic, autoimmune inflammatory disorder that primarily affects premenopausal women. Increased risk of atherosclerosis and cardiovascular death in patients with SLE was first described by Urowitz in 1976 (1), and subsequent studies have shown the association between SLE and accelerated rate of subclinical atherosclerosis (2, 3). The pathogenesis of atherosclerosis in SLE is a complex and multifactorial process. Although classical cardiovascular risk factors appear to have important roles in the development of cardiovascular disease in patients with SLE, there is still a 7.5-17-fold increased risk of cardiovascular events in this group of patients even after adjustment for classical cardiovascular risk factors (4, 5). Endothelial dysfunction (ED) is known to be early stage of atherosclerosis before the occurrence of atherosclerotic lesion formation and an independent predictor of cardiovascular events (6, 7). Previous data has well

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documented that patients with SLE have impaired endothelium-dependent vasodilation (8).

Oxidative stress, which enhances the modification of plasma lipids, is possibly associated with atherosclerotic events and coronary vasoreactivity. Cells have a comprehensive set of antioxidant defense mechanisms to prevent reactive oxygen species (ROS) formation and to limit their damaging effects. All serum antioxidants are usually measured together as the total antioxidant status (TAS). TAS gives the sum total of both exogenous as well as endogenous antioxidants. Thus, it gives the complete picture of the antioxidant status. Increased oxidative stress, an imbalance of nitric oxide and ROS, has been proposed to contribute to the development of ED and atherosclerosis in patients with SLE (9).

Because patients with SLE are at increased risk for cardiovascular complications, early detection of subclinical atherosclerosis and understanding the mechanisms of its development are of paramount importance. Although the relationship between oxidative stress and ED has been established in patients with SLE (10, 11), there is not much data regarding the relationship between TAS and ED in this patient population. Therefore, we aimed to evaluate the association between ED as assessed by flow-mediated dilation (FMD) and serum TAS, which is defined as all serum antioxidants, in patients with SLE.

Methods

Study design

This was an observational cross-sectional study.

Study population

Thirty-four patients with SLE and 39 age-, gender-, and body mass index (BMI)-matched healthy subjects were included in this cross-sectional study. Patients who fulfilled at least 4 classification criteria (2012 revised criteria) for SLE and who were not undergoing any treatment were recruited from the rheumatology outpatient clinic of Dr. Ersin Arslan Hospital and 25 Aralik State Hospital between May 2012 and April 2013 (12). All patients in our study were selected from those who were in a stable clinical condition (no need for immunosuppressive therapy intensification, i.e., current immunosuppressive drug dose increase or introduction of an additional immunosuppressive drug within the last 3 months). Patients with coronary artery disease, diabetes mellitus, hypertension, hyperlipidemia, smoking, alcohol intake, vasoactive drug use, moderate-to-severe valvular heart disease, left ventricular dysfunction (ejection fraction of <40%), hepatic or renal insufficiency (alanine aminotransferase and aspartate aminotransferase levels of >2-fold normal; serum creatinine levels of >1.5 mg/dL), hypothyroidism or hyperthyroidism, current acute or chronic infection, malignancy, BMI greater than 35 kg/m^2, or left ventricular mass index (LVMI) of ≥125 g/m^2 for men and ≥110 g/m^2 for women were excluded from the study. The study was conducted according to the recommendations set forth by the Declaration of Helsinki on Biomedical Research Involving Human Subjects. Written informed consent was obtained from each subject, and the institutional Ethics Committee approved the study protocol.

Study protocol

The study population consisted of 34 patients with SLE. After diagnosis of SLE, baseline clinical and demographic variables, including age, sex, SLE disease activity index (SLEDAI), BMI, duration of SLE, systolic and diastolic blood pressures (BPs), and heart rate, were recorded. Doppler ultrasound examination of the brachial artery for assessment of ED and blood sampling for assessment of TAS levels were performed in each study participant. The control group consisted of 39 individuals without any overt cardiovascular disease. They also underwent the same protocol including Doppler ultrasound examination and blood sampling.

Study variables

Assessment of endothelium-dependent vasodilation

All subjects rested for at least 15 min in a supine position in a quiet, dark, air-conditioned room (22°C-25°C) before any measurement. The subjects were instructed to fast for at least 8 h before undergoing FMD and to abstain from exercising, smoking, and ingesting alcohol and caffeine, all of which may affect FMD measurements. The brachial artery diameter was measured in the antecubital fossa just before it divided into branches using a Doppler ultrasound system with a high-resolution 7.5-MHz linear array transducer (Toshiba Applio 80, Tokyo, Japan). When a satisfactory transducer position with clear anterior and posterior intimal interfaces between the lumen and vessel wall was found, the surface of the skin was marked and all measurements were obtained from the same area. After acquiring a baseline rest image, a sphygmomanometer (BP) cuff was placed and inflated to 250 mm Hg (or at least 50 mm Hg above systolic BP) to occlude inflow in the brachial artery for 5 min. Reactive hyperemia was then created in the brachial artery after sudden cuff release. The percent change in FMD was estimated as the percent change in the vessel diameter over the baseline value at maximum dilation during reactive hyperemia and FMD was calculated according to this formula: FMD=100×(maximum diameter after hyperemia-baseline diameter/baseline diameter) (13). Only one measurement was performed. The intraobserver and interobserver variability for the measurement of FMD was 5.8% and 7.8%, respectively.

TAS measurement

Blood samples were obtained by direct venipuncture from the antecubital vein at 8:00 am after a 12-h overnight fast. Plasma for glucose, high-sensitivity C-reactive protein (hs-CRP), and lipid levels was analyzed on the same day, whereas plasma for TAS levels was stored at -80°C until the assay was performed. Serum TAS levels were measured using a TAS kit (Randox Labs, Crumlin, UK) and expressed as millimoles per liter

Table 1. Baseline characteristics of the study population

Variables	SLE group (n=34)	Control group (n=39)	*P
Age, years	37.2±11.0	35.9±6.1	0.54
Women/men	19/15	21/18	0.8
BMI	26.7±3.9	27.2±1.8	0.47
SLEDAI score	6.26±1.48	-	<0.0001
Disease duration, months	65 (1-360)	-	<0.0001
Systolic BP, mm Hg	119±10	119±12	0.81
Diastolic BP, mm Hg	74±6	76±10	0.33
Heart rate, bpm	74.0±3.8	73.1±10.9	0.64
Ejection fraction, %	68.1±3.6	66.9±2.5	0.09
Total cholesterol, mg/dL	175.2±40.6	180.6±29.5	0.54
HDL cholesterol, mg/dL	43.3±8.7	42.4±9.3	0.70
LDL cholesterol, mg/dL	108.1±28.3	109.5±22.3	0.82
Triglyceride	116.7±67.1	136.2±61.7	0.24
Glucose, mg/dL	93.4±7.0	91.7±5.7	0.26
Hemoglobin, g/dL	14.1±1.5	14.2±1.1	0.79
TAS, mmol/L	1.60±0.11	1.73±0.15	<0.001
hs-CRP, mg/L	8.2±6.0	2.9±4.0	<0.001
Basal diameter, mm	39.8±5.6	38.7±4.0	0.34
Hyperemic diameter, mm	42.9±5.7	42.7±4.3	0.83
FMD percent	8.1±4.9	10.6±4.7	0.04

Data are presented as mean±SD and number
*unpaired Student's t-test and chi-square test
BMI - body mass index; BP - blood pressure; FMD - flow-mediated dilation; HDL - high-density lipoprotein; hs-CRP - high-sensitivity C-reactive protein; LDL - low-density lipoprotein; SLE - systemic lupus erythematosus; SLEDAI - systemic lupus erythematosus disease activity index; TAS - total antioxidant status

(mmol/L). This assay relies on the ability of antioxidants in the sample to inhibit the formation of ABTS+ from oxidation of ABTS [2,2'-azino-di-(3-ethylbenzthiazoline sulfonate)] by metmyoglobin (a peroxidase). An antioxidant of known concentration (1.65 mmol/L) was used as a standard for the calculation of antioxidant levels in the samples. Fasting blood glucose, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein cholesterol, triglyceride, and hs-CRP levels were measured by spectrophotometric methods using an autoanalyzer (Architect c8000, Abbott, IL, USA).

Statistical analysis

Statistical analyses were performed with SPSS version 17.0 (SPSS, Chicago, IL, USA). All results are expressed as mean and standard deviation for normally distributed continuous variables. For categorical variables, numbers are used. All data were tested for normal distribution using the Kolmogorov-Smirnov test. Categorical data were compared between groups using the chi-square test or Fisher's exact test where appropriate. Parametric continuous data were compared between groups using unpaired Student's t-test and nonparametric data using the Mann-Whitney U test. Pearson's or Spearman's correlation analysis was used to test correlations where appropriate. To determine the independent predictors of FMD response, multiple linear regression analysis was used, in which FMD was entered as the dependent variable and TAS, hs-CRP, and SLEDAI were entered as the independent variables. All p values are two-tailed and a value of $p<0.05$ was considered to be statistically significant.

Table 2. Correlations between FMD percent and biochemical parameters in the study groups

	FMD			
	SLE group		Control group	
Variable	r	*P	r	*P
TAS	0.448	0.008	0.367	0.03
hs-CRP	-0.368	0.04	-0.320	0.07
Glucose	-0.108	0.56	-0.150	0.40
Total cholesterol	0.020	0.93	-0.192	0.28
LDL cholesterol	0.096	0.64	-0.234	0.19
HDL cholesterol	-0.004	0.98	-0.006	0.97
Triglyceride	-0.208	0.31	-0.114	0.52
Systolic BP	-0.078	0.66	0.053	0.77
Diastolic BP	-0.091	0.60	-0.039	0.82
SLEDAI score	-0.365	0.03		
Disease duration	0.183	0.32		

*Pearson's correlation analysis
BP - blood pressure; FMD - flow-mediated dilation; HDL - high-density lipoprotein; hs-CRP - high-sensitivity C-reactive protein; LDL - low-density lipoprotein; SLE - systemic lupus erythematosus; SLEDAI - systemic lupus erythematosus disease activity index; TAS - total antioxidant status

Results

The baseline characteristics and clinical data of 34 patients with SLE (19 women and 15 men; mean age: 37.2±11.0 years) and 39 control subjects without SLE (21 women and 18 men; mean age: 35.9±6.1 years) are summarized in Table 1. There were no statistically significant differences between the groups with regard to age, sex, BMI, mean heart rate, systolic and diastolic BPs, lipid profiles, and hemoglobin and glucose levels.

The mean TAS level were found to be significantly lower in patients with SLE than in controls (1.60±0.11 vs. 1.73±0.15 mmol/L; $p<0.001$) (Table 1). In addition, hs-CRP levels were significantly higher in patients with SLE than in controls (8.2±6.0 vs. 2.9±4.0 mg/dL; $p<0.001$) (Table 1). Baseline and hyperemic diameters were similar between the groups. However, the FMD percent was significantly lower in patients with SLE than in controls (8.1±4.9 vs. 10.6±4.7, $p=0.04$) (Table 1).

Correlation analysis showed a significant moderate positive correlation between FMD and TAS in the SLE group ($r=0.448$, $p=0.008$) and the control group ($r=0.367$, $p=0.03$) and a significant moderate negative correlation between FMD and serum hs-CRP ($r=-0.368$, $p=0.04$) and between FMD and SLEDAI ($r=-0.365$, $p=0.03$) in only the SLE group (Table 2 and Fig. 1). TAS, hs-CRP,

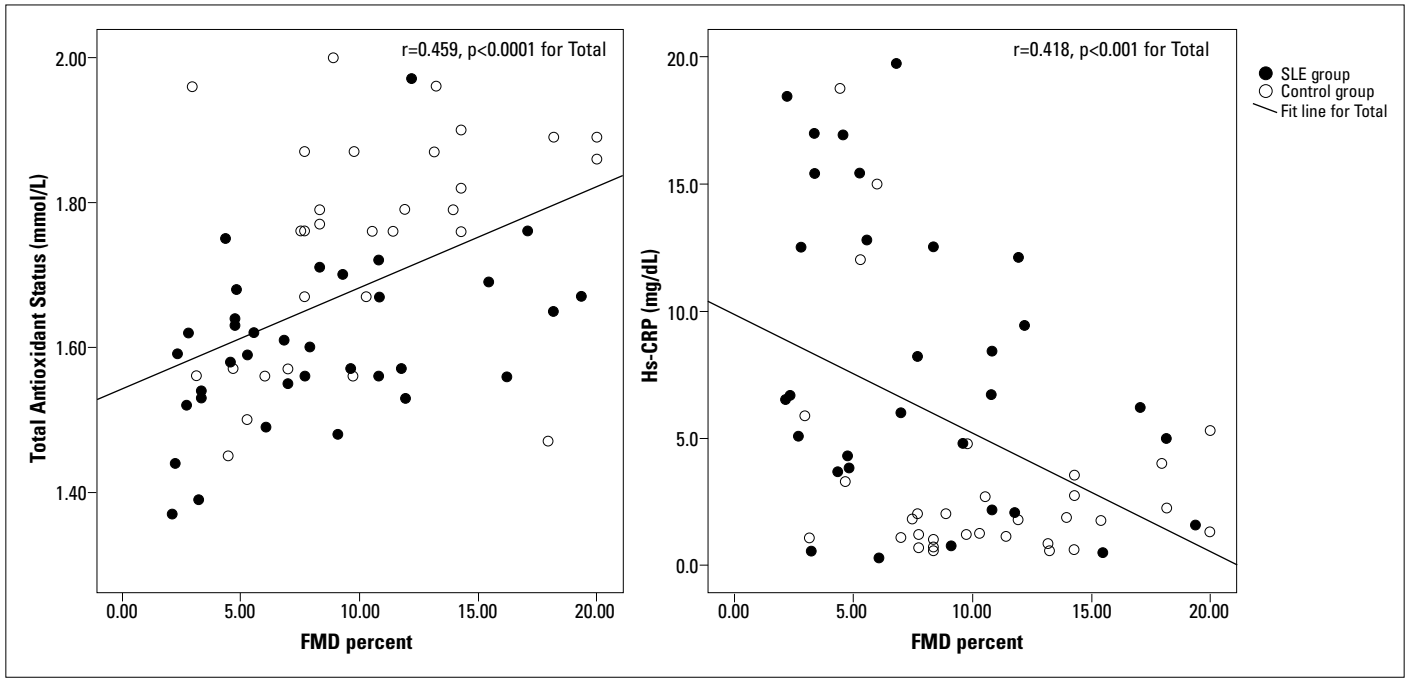

Figure 1. Correlations between FMD, TAS, and hs-CRP in the study groups
FMD - flow-mediated dilation; hs-CRP - high-sensitivity C-reactive protein - TAS: total antioxidant status

Table 3. Multiple linear regression analysis of the factors affecting FMD

Variable	β	95% CI	*P
TAS	0.50	7.836-34.025	0.003
hs-CRP	-0.33	-0.530-0.017	0.03
SLEDAI	0.06	-0.038-0.131	0.03

*Multiple linear regression analysis
In regression analysis: independent variables: TAS, hs-CRP, SLEDAI; dependent variable: FMD
FMD - flow-mediated dilation; hs-CRP - high-sensitivity C-reactive protein; SLEDAI - systemic lupus erythematosus disease activity index; TAS - total antioxidant status

and SLEDAI were independent determinants in regression analysis (β=0.50, p=0.003; β=-0.33, p=0.03; and β=0.06, p=0.03; respectively) affecting FMD (Table 3).

In addition, patients with SLE were further divided into 2 groups according to anticardiolipin antibody (aCLA) positivity. As can be seen in Table 4, only hs-CRP levels were higher in the aCLA-positive subgroup (13.8±4.3 vs. 5.6±4.8 mg/dL; p<0.001). Although FMD was decreased in the aCLA-positive subgroup in comparison with the aCLA-negative subgroup, the difference was not statistically significant (5.6±2.4 vs. 9.4±5.4, p=0.06).

Discussion

Our study revealed that patients with SLE have decreased TAS levels, diminished FMD, and increased hs-CRP levels compared with healthy controls. A novel finding of this study is that TAS, hs-CRP, and SLEDAI were independently associated with ED in patients with SLE.

Patients with SLE are at increased risk for developing accelerated atherosclerosis. More than one-third of all SLE deaths occur among persons younger than 45 years. In recent years, the life expectancy and prognosis of patients with SLE have improved; however, deaths from cardiovascular diseases have increased (1). Although immune dysregulation and chronic inflammation in the pathogenesis of atherosclerosis has been well established, the reason for premature and accelerated atherosclerosis in SLE is still debatable and not fully understood (14).

In the present study, we found lower TAS levels in patients with SLE when compared with healthy controls. In addition, there was a positive correlation between TAS and FMD. It is well known that there is a significant increase in oxidative stress in patients with SLE resulting from an imbalance between elevated ROS and reduced antioxidant status (6, 7). Previous data have also shown that increased oxidative stress is positively correlated with disease activity in patients with SLE (15). Oxidative stress resulting from excess free radical production and reduced antioxidant status, as documented in our study, seen in SLE causes lipid peroxidation and can promote inflammatory processes, exacerbating inflammation and affecting tissue damage, and may contribute to atherogenesis (16-18). In a previous work by Delgado Alves et al. (19), it was shown that paraoxonase, an enzyme with antioxidant activity preventing LDL oxidation, was reduced in patients with SLE. Paraoxonase activity was positively correlated with TAS levels. They speculated that these interactions may be related to the development of atherosclerosis in SLE. In a recent study, Yılmaz et al. (20) reported that coronary flow reserve (CFR), which reflects coronary microvascular function, and serum TAS levels were significantly low in patients with SLE. They also showed that CFR was positively correlated with serum TAS levels. The findings of these studies indicate

Table 4. Baseline characteristics of the study population according to the presence of aCLA

Variables	aCLA-positive group (n=11)	aCLA-negative group(n=23)	*P
Age, years	35.4±10.6	38.0±11.3	0.47
SLEDAI score	7.00±0.89	5.91±1.60	0.05
Disease duration, months	48 (21–126)	42 (24–84)	0.78
BMI	26.9±4.1	26.6±3.9	0.42
Systolic BP, mm Hg	116±9	120±11	0.51
Diastolic BP, mm Hg	75±8	74±6	0.66
Heart rate, bpm	74.6±3.2	73.7±4.1	0.44
Ejection fraction, %	68.1±3.2	68.2±3.8	0.90
Total cholesterol, mg/dL	175.9±29.0	174.9±45.9	0.88
HDL cholesterol, mg/dL	43.3±8.7	42.4±9.3	0.17
LDL cholesterol, mg/dL	108.1±28.3	109.5±22.3	0.51
Triglyceride, mg/dL	116.7±67.1	119.2±61.7	0.23
TAS, mmol/L	1.59±0.07	1.61±0.13	0.69
hs-CRP, mg/L	13.8±4.3	5.6±4.8	<0.001
FMD percent	5.6±2.4	9.4±5.4	0.06

Data are presented as mean±SD and number or median with the 25th and 75th percentile
*unpaired Student's t-test, chi-square test, and Mann–Whitney U test
aCLA - anticardiolipin antibody; BMI - body mass index; BP - blood pressure; FMD - flow-mediated dilation; HDL - high-density lipoprotein; hs-CRP - high-sensitivity C-reactive protein; LDL - low-density lipoprotein; SLE - systemic lupus erythematosus; SLEDAI - systemic lupus erythematosus disease activity index; TAS - total antioxidant status

that low antioxidant levels may be correlated with atherogenesis in patients with SLE; our results are also consistent with these findings.

ED, the early stage of atherosclerosis that predicts future cardiovascular events, may have a role in the development of premature atherosclerosis in patients with SLE (21). FMD is a non-invasive technique that detects ED by measuring the change in the brachial artery diameter in response to reactive hyperemia (22). In the present study, we found that FMD was significantly lower in patients with SLE than in controls, reflecting the impairment of endothelial function in this group of patients. Furthermore, there was a positive correlation between FMD and TAS. Previous studies have examined various factors associated with impaired FMD in patients with SLE. El-Magadmi et al. (23) demonstrated that FMD was impaired in patients with SLE. In that study, systolic BP was significantly associated with impaired FMD and carotid intima-media thickness showed a negative correlation with FMD in patients with SLE. In a recent meta-analysis, Wang et al. (21) showed that FMD was significantly impaired in patients with SLE, even in the subgroup of patients without cardiovascular risk factors. Piper et al. (24) also reported that patients with SLE showed significant impairments in endothelial function compared with healthy controls and total cholesterol levels were inversely correlated with endothelial function in patients with SLE. In our study, we did not find any significant correlations between FMD percent and glucose levels, lipid parameters, and BP. This was possibly due to exclusion of patients with diabetes, hyperlipidemia, and hypertension from the study. Impaired endothelial function in patients with SLE may suggest that this group of patients have an increased risk of atherosclerosis and future adverse cardiac events.

It is well known that hs-CRP is a marker of systemic microinflammation and patients with SLE have increased hs-CRP levels (25). Consistent with previous data, hs-CRP levels were significantly higher in the SLE group than in healthy controls in our study (25-27). Furthermore, increased hs-CRP levels were noted in SLE patients with positive aCLA. Biologically, aCLA is considered to be an indicator of vascular endothelial and endomyocardial damage (28). Considering that vascular inflammation is the key feature of atherosclerotic lesion formation and progression, this finding may suggest that patients with SLE, particularly those who are positive for aCLA, have ongoing low-grade inflammation or microinflammation and therefore have an increased risk of future cardiovascular events (29). In addition, we found a negative correlation between FMD and hs-CRP in our study. In agreement with our study, Karadağ et al. (30) demonstrated an inverse relationship between CRP levels and FMD in patients with SLE.

Study limitations

We recognize that our study has limitations that warrant consideration. The cross-sectional design does not allow us to infer a causal relationship between FMD percent and TAS and hs-CRP levels. We did not standardize all factors influencing oxidative stress such as lifestyle, nutritional habits, and physical stress conditions, and we did not measure other inflammatory markers such as tumor necrosis factor alpha, complement fractions, anti-dsDNA positivity, LAC positivity, and interleukin-6. Our study population was small and we included only stable patients who were not undergoing any treatment. We did not measure the total oxidant status in the study groups. Further study is needed to examine the relationship between SLE and TAS.

Conclusion

In this study, serum TAS levels, FMD, and hs-CRP levels were studied together in patients with SLE. The results indicate that reduced serum TAS levels, impaired FMD, and elevated hs-CRP levels may contribute to the development of ED in patients with SLE, which may eventually lead to atherosclerosis. Further investigations are required for understanding the mechanisms underlying atherosclerosis in SLE.

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