Evaluation of Ischemia Modified Albumin Levels and Carotid Intima Media Thickness in Patients with Systemic Lupus Eythematosus

Sami Küçükşen[1], Aysun Toker[2], Adem Küçük[3], Ender Salbaş[1], Ramazan Uçar[4], Mehmet Kayrak[5], Recep Tunç[3]

Necmettin Erbakan University,
Meram Medical Faculty,

[1]Department of Physical Medicine and Rehabilitation,

[2]Department of Biochemistry,

[3]Department of Rheumatology,

[4]Departmant of Internal Medicine,

[5]Department of Cardiology

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Correspondence (Yazışma Adresi):
Doç. Dr. Aysun Toker,
Necmettin Erbakan University, Meram Medical Faculty, Department of Biochemistry, Meram/Konya
e-mail: aysuntoker@gmail.com
Phax: +90 332 2236225

ABSTRACT

Systemic lupus erythematosus (SLE) is a major cause of cardiovascular mortality and morbidity associated with early atherosclerosis. Carotid intima-media thickness (CIMT) is one of the early markers of atherosclerosis. Oxidative stress plays an important role in pathophysiologic mechanisms of SLE. Ischemia modified albumin (IMA) and total oxidant status (TOS) have been associated with oxidative stress in several diseases. In our study we aimed to investigate the relationship between serum IMA and TOS levels with CIMT in patients with SLE and to compare these with control group values. In our study, 60 SLE patients diagnosed according to the American College of Rheumatology (ACR) criteria and 35 healthy controls were included. Serum IMA levels were measured by manually spectrophotometric method. Increased serum TOS and IMA levels and CIMT were found in patients with SLE when compared with control group ($p< 0.05$). However, we could not find any significant correlation between serum TOS and IMA levels with CIMT values in patient group ($p> 0.05$). Our results suggest that serum levels of IMA and TOS increase in SLE. However lack of association between the tested parameters suggests that further researches are needed.

Key Words: *Ischemia modified albumin, Systemic lupus erythematosus, Carotid intima media thickness, Total oxidant status*

Sistemik Lupus Eritematozis Hastalarında İskemik Modifiye Albumin Seviyeleri ve Karotis İntima Media Kalınlıklarının Değerlendirilmesi

ÖZET

Sistemik lupus eritamatozus (SLE)'da görülen kardiyovasküler mortalite ve morbiditenin başlıca nedenlerinden biri erken aterosklerozdur. Karotis intima media kalınlığı (KİMK) aterosklerozun erken belirteçlerinden biridir. Oksidatif stres SLE´un patofizyolojik mekanizmalarında rol oynamaktadır. İskemik modifiye albumin (İMA) ve total oksidan durum (TOS) pek çok hastalıkta oksidatif stres ile ilişkilidir. Çalışmamızda, SLE hastalarında serum İMA ve TOS seviyelerinin KİMK ile ilişkisini araştırmayı amaçladık. Çalışmamıza Amerikan Romatoloji Derneği kriterlerine göre tanı konan 60 SLE hastası ve 35 sağlıklı kontrol dahil edildi. Serum İMA seviyeleri manuel olarak spektrofotometrik metodla ölçüldü. Kontrol grubuna göre SLE hastalarında artmış serum İMA ve TOS seviyeleri ve artmış KİMK bulundu ($p< 0.05$). Bununla birlikte, hasta grubunda TOS ve İMA seviyelerinin KİMK ile ilişkisi saptanamadı ($p> 0.05$).

Sonuçlarımız SLE hastalarında artmış serum İMA ve TOS seviyelerini göstermektedir. Ancak çalışılan parametreler arası ilişkinin yokluğu nedeni ile ileriki çalışmalara ihtiyaç vardır.

Anahtar kelimeler: *İskemi modifiye albumin, Sistemik lupus eritamatozus, Karotis intima media kalınlığı, Total oksidan durum*

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease and affects several organs. Patients with SLE have substantially increased morbidity and mortality from coronary artery disease (CAD) (1). SLE could be an independent risk factor for the development of cardiovascular disease (CVD) (2). Several studies reported that traditional cardiovascular risk factors do not fully account for increased risk of CVD in this population (1,3). An increased risk for myocardial infarction and stroke after controlling for traditional factors was reported (4). Therefore some other factors such as inflammatory mechanisms inherent in SLE and treatment-related factor might play an important role in the atherosclerotic process. Some studies have determined the atherosclerosis in patients with SLE, focussing on a marker such as the mean carotid intima-media thickness (CIMT) evaluated by high-resolution ultrasonographic measurement (5).

Recently it was reported energy generating pathways, Krebs cycle, ß-oxidation of lipids and the pool of available amino acids in SLE. One of the important findings of this study was the reduced levels of glutathione (GSH) as a key intracellular antioxidant. The lipid peroxidation marker, serum malondialdehyde (MDA) levels were also found significantly higher in SLE patients than controls (6). There are findings indicate that increased oxidative stress and/or the reduced availability of antioxidants in SLE (7-9).

Ischemia-modified albumin (IMA) is a marker of tissue ischemia (10). One of the causes of IMA formation is reactive oxygen species (ROS) generated during ischemia. Therefore, IMA is postulated as a marker of oxidative stress (11). In a previous study, we demonstrated that serum IMA levels were positively correlated with CIMT, oxidative stres and inflammatory markers when all study groups were included in the model (12).

The present study was designed to investigate the association between serum IMA and total oxidant status (TOS) levels and subclinical atherosclerosis, as determined by CIMT, in patients with SLE.

MATERIAL AND METHOD

Subjects

The study population consisted of 60 patients with diagnosed SLE last 6 months and without any disease relapse within the last 3 months, fulfilling the American College of Rheumatology criteria for SLE (13). Disease activity was assessed by the use of the SLE Disease Activity Index (SLEDAI) (14). In patient group, patients in remission by SLEDAI score were studied. 35 control subjects matched for age, gender, without a previous history of CVD were selected from subjects recruited in outpatient clinic. All participants underwent a complete clinical and laboratory evaluation. Traditional risk factors for atherosclerosis, including hypertension, lipid abnormalities, diabetes mellitus, smoking status, body mass index (BMI), and family history of CVD were assessed. Pateints with consisted of known coronary disease or symptoms suggestive of CVD (angina, arrhythmia, congestive heart failure, stroke, and peripheral arterial disease) were excluded from study. BMI was measured in all participants and calculated as weight (in kilograms) divided by height (in meters) squared. The Ethical Committee approved the study and written informed consent was obtained from each participant.

Sample collection and analysis

An overnight (10 to 12 h) fasting venous blood samples were collected from the all subjects. Serum lipid profile, C reactive protein (CRP) levels were analyzed on Abbot Architect 16000 system with the original reagents. Low density lipoprotein (LDL) cholesterol levels were calculated with Friedewald formula. Complete blood counts were measured by the method of laser-based flow cytometric impedance, using an automated blood cell counter (Mindray BC-6800, Shenzhen, PR China). Whole blood erythrocyte sedimentation rate (ESR) was determined by iSed (Alcor Scientific).

Serum TOS levels were measured using commercially available kits (Rel Assay, Turkey). Measurement of TOS was performed by a method in which oxidants present in the sample oxidize the ferrous-ion-o-dianisidine complex to ferric ion. The ferric ion generates a colored complex in

an acidic medium. The color intensity was related to the total amount of oxidant molecules present in the sample. Results were expressed as μmol H2O2 equivalent/L.

Ischemia-modified albumin levels were measured using colorimetric method based on albumin cobalt binding test (10). Absorbance was measured at 470 nm in a spectrophotometer (UV1601; Shimadzu Scientific, Tokyo, Japan). The results were expressed in absorbance units (ABSU). All IMA measurements were done in the same run.

Carotid intima media thickness

A single investigator conducted CIMT in controls and SLE patients. The carotid arteries were evaluated with the Vivid 7 echocardiography device (General Electrics, Horten, Norway) by using a 10-MHz linear probe. The acquired images were recorded for playback analysis and were later measured off-line. The common carotid artery, the carotid bulb, and internal and external carotid arteries were visualized on both sides. The IMT of the carotid arteries were measured in the distal common carotid artery at a level 15 to 20 mm proximal to the carotid bulb. The two bright echogenic lines in the arterial wall were identified as the intima and the media. Three measurements were made for each side of the body; separate means were calculated and recorded as the right and left IMT.

Statistical analysis

SPSS 16.0 statistical package program was used for all data analyses. Kolmogorov Smirnov test was used before the analysis to determine groups distribution. The data distributed nonparametrically are presented as median (minimum-maximum) and the data distributed parametrically are presented as mean ± SD. Mann Whitney test or independent t-test were performed for comparisons between the patients and controls. Spearman's correlation analysis was used to evaluate the degree of association between two variables. The differences between the parameters was considered significant at a probability level of $p<0.05$.

RESULTS

The demographic characteristics and biochemical analyses of the subjects included in the study were given in Table 1. There were no significant differences in terms of age, gender, BMI and serum lipid profile between SLE and the control group ($p> 0.05$). Serum CRP and ESR levels were significantly higher in patient group than controls as expected ($p< 0.01$). Increased serum TOS and IMA levels were found in patients with SLE when compared with control group ($p< 0.05$). Moreover patients with SLE had significantly higher CIMT values than same parameter of controls at the time of sampling ($p< 0.05$).

Simple correlation analyses were performed to investigate the association of biochemical analyses with BMI, CIMT in both groups and in patient group. Serum total cholesterol and LDL cholesterol levels were positively

***Table 1**. The demographic characteristics and biochemical analyses of the all study participants.*

Parameters	SLE Group (n= 60)	Control Group (n= 35)	P values
Age (Years)	40.64±10.61	38.73±9.81	p> 0.05
Gender (F/M)	55/5	30/5	
BMI (kg/m2)	27.18±4.84	26.34±4.46	p> 0.05
WBC (count/mm3)	6.48±2.60	6.94±1.26	p> 0.05
Triglyceride (mg/dL)	114.38±72.72	136.95±69.18	p> 0.05
Total cholesterol (mg/dL)	171.82±37.02	175.36±30.92	p> 0.05
LDL cholesterol (mg/dL)	99.04±27.76	100.12±27.93	p> 0.05
CRP (mg/L)	5.03 (1.16-83.00)	2.43 (1.00-11.00)	p< 0.01
ESR (mm/h)	28 (2-107)	4.50 (2-23)	p< 0.01
CIMT (mm)	0.7 (0.3-1.2)	0.6 (0.4-0.9)	p< 0.05
TOS (μmol H2O2 equivalent/L)	5.55 (2.92-58.8)	4.58 (2.73-15.40)	p< 0.05
IMA (ABSU)	0.64±0.16	0.48±0.14	p< 0.05

BMI: body mass index, WBC: white blood cell, CRP: C reactive protein, ESR: erythrocyte sedimentation rate, CIMT: carotid intima-media thickness, TOS: total oxidative stress, IMA: ischemia-modified albumin

correlated with CIMT in patients with SLE (r= 0.353, p<0.01 and r= 0.293, p< 0.05 respectively). We found age was correlated with CIMT in this group (r= 0.531, p< 0.05). However, we could not find any significant correlation between serum TOS and IMA levels with CIMT values in the same group (p> 0.05).

DISCUSSION

In our study the CIMT and serum IMA and TOS levels were significantly increased in patients with SLE relative to control group. In SLE group, CIMT correlated with age, total cholesterol and LDL cholesterol levels.

It is known that SLE is a systemic inflammatory disease and is also associated with accelerated atherosclerosis (15). SLE is associated with increased mortality as a reason of premature atherosclerosis, which cannot be entirely explained by traditional risk factors (16). But atherosclerosis seen in patients with SLE are extremely complex. Conventional risk factors such as age, family history and smoking play important roles in CVD process. However these factors cannot entirely explain the increased rate (17). Interestingly, arterial stiffness is increased in SLE patients despite a low risk for CVD according to Framingham score (18). In SLE, autoantibodies and sensitized lymphocytes against self-antigens facilitate the entrance of monocytes into blood vessel walls. The monocytes may activate endothelial cells and cause the atherosclerosis formation (19). Assessment of CIMT by ultrasonography could be a direct measurement of systemic atherosclerosis. Therefore, it could be a sensitive marker for the earliest stages of atherosclerosis (20). It was showed that CIMT determination is predictive of future cardiovascular events in women with SLE without previous history. So the carotid ultrasound could provide an additional tool for risk stratification in patients with SLE (21). In our study we found increased CIMT in SLE group than control group. Jung et al. reported increased CIMT was correlated with age in SLE patients (22). We also found significant correlation between CIMT and age in patients with SLE. Our results are harmonies with literature findings (22,23).

Recently, it was reported that patients with SLE who have no overt cardiovascular disease are at increased risk for endothelial dysfunction. The authors concluded that this may be associated with underlying inflammation and impairment of antioxidant status (24). Considerable clinical studies in SLE implicate the role of oxidative stress in the pathogenesis of SLE. A strong inflammatory component and chronic overproduction of ROS and reactive nitrogen species play important roles in underlying mechanisms. Immune cell dysfunction, autoantigen production, and autoantibody reactivity could result from oxidative stress in SLE pathogenesis (25). Several studies reported increased oxidative stress and decreased antioxidant enzyme activities in SLE (7,26,27). Shah et al (26) reported higher lipid peroxidation levels, measured as MDA, lower antioxidant molecule GSH and lower antioxidant enzyme levels measured as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) in SLE patients as compared to controls. Taysi et al. (27) found higher MDA, ceruloplasmin, SOD activitiy and lower GPX, CAT activities and transferrin levels in serum of SLE patients compared with controls. Moreover, positive correlation between MDA levels and SLEDAI was found (26,27).

The decreased antioxidant enzyme activity might result from the allelic variations in enzyme gene (28). But the exact mechanism is stil unclear. It is also concluded that increased MDA modified proteins, antibodies aganist antioxidant enzymes, in patients with SLE are associated with disease activity in patients with SLE (29,30). Moreover, enhanced oxidative stress response is associated with higher SLEDAI scores (26,31). In this study we found higher serum TOS levels in patients with SLE than control group.

A novel serum biomarker IMA is considered to be related with ROS which may chemically modify human serum albumin (11). Although the exact mechanism is not exactly known, hypoxia, acidosis, free radical injury may play roles in IMA formation (32,33). IMA has been reported as an oxidative stress marker in different pathologies (34-36). There several studies which investigate IMA levels in different rheumatologic diseases (37,38). Capkin et al. (38) reported higher serum MDA, TOS and IMA levels in the SLE patients compared with the control group. Interestingly authors suggested that higher IMA levels could be used as a significant tool in distinguishing vascular involvement in particular. We could not find any knowledge about IMA levels in patient with SLE.

Our results suggest that serum levels of IMA and TOS increase in SLE. However lack of association between the tested parameters suggests that further researches are needed.

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