ENDOTHELIAL DYSFUNCTION IN SYSTEMIC *LUPUS ERYTHEMATOSUS*

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Abstract. Systemic *lupus erythematosus* (SLE) is associated with an increased risk of atherosclerosis; endothelial dysfunction representing the first step in its pathogenesis. The aim of this study is represented by the assessment of the endothelial dysfunction in SLE and the characterization of SLE specific factors which contribute to its appearance. The study was done on 24 subjects, divided into two groups: group A (12 patients with SLE without renal involvement) and group B (12 healthy sex and age-matched controls). Total cholesterol, triglycerides, antinuclear antibodies, anti dsDNA antibodies, C_3, circulating immune complexes were determined in all patients. SLE activity was assessed using SLE Disease Activity Index (SLEDAI). Endothelial function was assessed by means of flow mediated dilation (FMD) on brachial artery, using B-mode ultrasonography. The statistical analysis was done using Pearson's test and Student's t-test. $p < 0.05$ was considered statistically significant. The group of SLE patients was formed of 12 females, with the mean age of 37.16 ± 9.69 years. The values of SLE specific tests and SLEDAI were represented by: anti dsDNA antibodies 1/682 ± 1/914, C_3 68.91 ± 11.91 mg/dL, circulating immune complexes 10.03 ± 2.85 μEq/mL, total cholesterol 208.66 ± 49.63 mg/dL, triglycerides 153.41 ± 46.26 mg/dL, SLEDAI 11.66 ± 3.70. The values of FMD were 8.85 ± 2.02% (group A) and 20.33 ± 6.19% (group B), $p < 0.001$. The statistical analysis showed a strong inverse correlation between FMD and SLEDAI, a strong correlation between FMD and C_3, respectively anti dsDNA antibodies, a moderate inverse correlation between FMD and circulating immune complexes, total cholesterol, systolic and diastolic blood pressure. Endothelial dysfunction is present in SLE patients even in the absence of traditional cardiovascular risk factors, due to disease activity.

Key words: endothelial dysfunction, systemic *lupus erythematosus*.

INTRODUCTION

Systemic *lupus erythematosus* (SLE) represents the autoimmune disease, with a wide range of clinical and biological manifestations [8]. Despite the improvement of therapeutic regimes, the morbidity and mortality associated with SLE remained at high levels. In 1976, Urowitz *et al*. [18] postulated a bimodal

Received: May 2008;
in final form June 2008.

ROMANIAN J. BIOPHYS., Vol. 18, No. 3, P. 237–244, BUCHAREST, 2008

mortality pattern in patients with this disease: in the first part of evolution, mortality is due to severe infections or to disease activity, but after 5 years of SLE course, mortality is caused by the accelerated atherosclerosis and its consequences.

During the last 3 decades, several authors studied the atherosclerosis in lupus. It was proved that atherosclerosis has a high incidence among young women with SLE. These patients have a high prevalence of coronary artery disease and an incidence of myocardial infarction up to 50 times higher than age-matched healthy subjects. This high incidence of atherosclerosis in SLE cannot be attributed only to traditional risk factors [3, 11].

In healthy subjects, endothelium is not a simple physical barrier between the blood flow and the underlying tissues. This structure has many functions, like: continuous regulation of vascular tone, leucocytes adhesion, maintenance of the balance between thrombotic and anticoagulant properties of the blood [12]. When these functions of the endothelium are affected, endothelial dysfunction appears. Endothelial dysfunction is considered the first step in the atherogenetic process; it was identified even in patients with SLE, without cardiovascular risk factors [9]. Endothelial dysfunction in SLE is produced by the clustering of traditional risk factors, adverse effects of treatment and SLE itself as an independent risk factor [1, 14]. Systemic inflammation, autoantibodies directed to double stranded DNA (dsDNA), ribonucleoproteins (nRNP), endothelial cells, phospholipids, circulating immune complexes, activated complement products, lupus nephropathy, dyslipidemia represent some factors related to SLE which contribute to appearance of endothelial dysfunction [7, 19].

A non-invasive method for the assessment of endothelial function is flow mediated vasodilation (FMD) on brachial artery, using vascular ultrasonography.

The aim of this study is represented by the assessment of endothelial dysfunction in SLE patients using vascular ultrasonography and the characterization of SLE specific factors which contribute to its appearance.

MATERIALS AND METHODS

The study was performed on two groups of subjects: group A, formed by 12 patients with SLE without renal involvement and group B, formed by 12 healthy sex and age-matched controls. The diagnosis of SLE was established based on American College of Rheumatology criteria. SLE treatment consisted of prednisone +/– azathioprine.

The procedures followed were in accordance with the ethical standards of the Hospital Ethics Commitee and with the Helsinki Declaration of 1975, as revised in 2000 (5)

Total cholesterol (Abbott photometry), triglycerides (Abbott reactive), antinuclear antibodies (immunofluorescence on Hep-2 cells), anti dsDNA antibodies (immunofluorescence on Crithidia luciliae), C_3 (Roche immunoturbidimetry), circulating immune complexes (EIA method) were determined in all patients.

The SLE activity was assessed using SLE Disease Activity Index (SLEDAI).

Endothelial function was assessed by means of flow-mediated vasodilation on brachial artery, using B-mode ultrasonography (ALOKA ProSound 4000, with linear transducer of 7.5 MHz). Before the test, the patient was relaxed in a stable room temperature between 20 – 25 ºC; the smoking was prohibited. The diameter of brachial artery was measured incident with the R wave of the electrocardiograph trace (Di). Then, ischemia was induced by inflating the pneumatic cuff to a pressure 50 mmHg above systolic one, in order to obliterate the brachial artery and induce ischemia. After 5 minutes, the cuff was deflated and the diameter was measured after 60 seconds post-deflation (Df). FMD was calculated with the formula:

$$FMD = [(Df - Di)/Di] \times 100 \quad (1)$$

All the values were presented as mean ± standard deviation. The statistical analysis was done using Pearson's test (for correlation) and Student's t-test (for comparing of FMD between the two groups). $p < 0.05$ was considered statistically significant.

RESULTS

The demographic, clinical and biological characteristics of the studied groups are shown in Table 1. Diabetes mellitus and chronic renal diseases were absent in all subjects.

Table 1

Characteristics of the studied groups

Parameter	SLE group	Control group
Males/Females	0/12	0/12
Mean age (years)	37.16 ± 9.69	35.02 ± 8.21
Mean duration of SLE evolution (years)	7.16 ± 3.66	0
Total cholesterol (mg/dL)	208.66 ± 49.63	209.25 ± 35.27
Triglycerides (mg/dL)	153.41 ± 46.26	155.71 ± 25.87
Smoking (%)	41.66%	33.33%
Blood pressure (mmHg)	137.08 ± 19.59/82.91 ± 9.87	135.94 ± 8.99/80.75 ± 9.29

The results of SLE specific tests and SLEDAI are presented in Table 2.

Table 2

SLE specific parameters

Parameter	**Mean value ± SD**
Anti dsDNA antibodies	1/682 ± 1/914
C_3 (mg/dL)	68.91 ± 11.91
Circulating immune complexes (μEq/mL)	10.03 ± 2.85
SLEDAI	11.66 ± 3.70

In SLE group, FMD was lower than in the control group (Table 3).

Table 3

FMD in the studied groups

	SLE group	**Control group**	***p***
FMD (%)	8.85 ± 2.02	20.33 ± 6.19	< 0.001

The impaired endothelial function, assessed by vascular ultrasonography on brachial artery, is presented in Figures 1, 2 (FMD = 8.57 %).

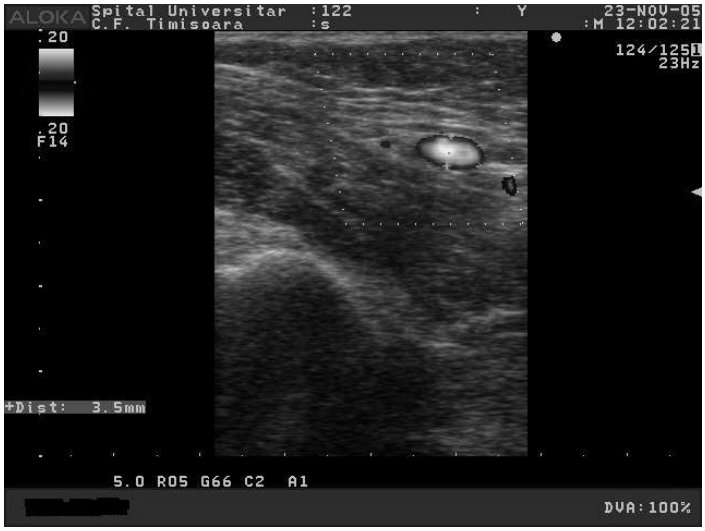

Fig. 1. Brachial artery diameter before FMD test.

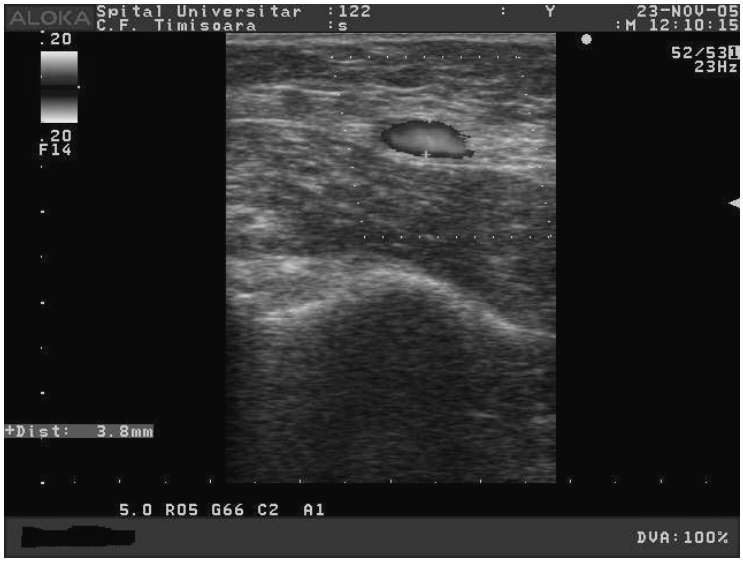

Fig. 2. Brachial artery diameter after FMD test.

The correlations between FMD and biological and immunological parameters are shown in Table 4.

The statistical analysis showed: a strong inverse correlation between FMD and SLEDAI, a strong correlation between FMD and C_3, anti dsDNA antibodies, a moderate inverse correlation between FMD and circulating immune complexes, total cholesterol, systolic and diastolic blood pressure.

Table 4

Correlations between FMD and SLE parameters

Parameter	**Correlation coefficient**
SLEDAI	$r = -0.7321$, $p < 0.001$
C_3	$r = 0.7117$, $p < 0.001$
Circulating immune complexes	$r = -0.4891$, $p < 0.01$
Anti dsDNA antibodies	$r = 0.7201$, $p < 0.001$
Total cholesterol	$r = -0.4450$, $p < 0.01$
Systolic blood pressure	$r = -0.4358$, $p < 0.01$
Diastolic blood pressure	$r = -0.4203$, $p < 0.01$

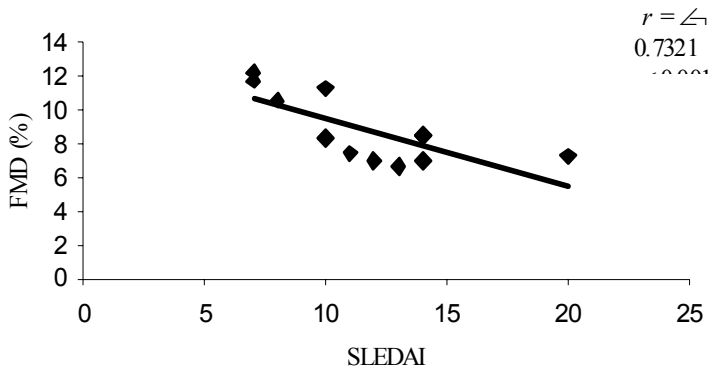

Fig. 3. Correlation between FMD and SLEDAI.

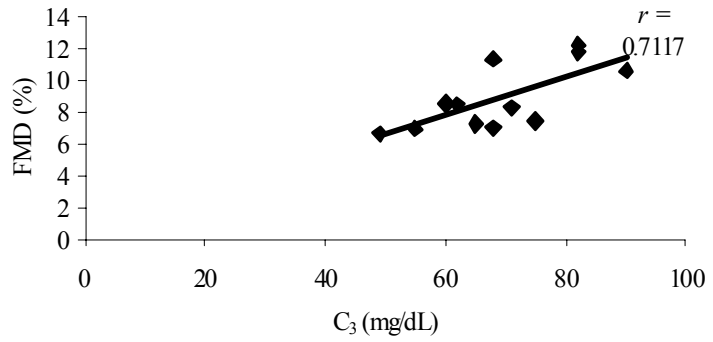

Fig. 4. Correlation between FMD and C_3.

DISCUSSIONS

The patients with SLE have a high incidence of atherosclerosis with its main consequence: coronary artery disease. Epidemiological studies have shown that SLE women aged 35 – 44 years were over 50 times more likely to develop myocardial infarction than women of similar age from general population [11]. Anatomo-pathological investigations have revealed that the SLE patients were prone to develop a premature atherosclerosis [2]. The increased risk of atherosclerosis is not exclusively related to traditional risk factors alone [9]. In the last years, SLE itself appeared like an independent risk factor for atherosclerosis, acting through autoimmune vascular injury [14].

In patients with systemic *lupus erythematosus*, atherosclerosis has a long period of subclinical evolution. The first reversible step in the atherogenesis process is represented by the endothelial dysfunction [16]. Endothelial dysfunction

appears when the normal functions of the endothelial cells (control of vascular tone and blood pressure, regulation of leucocytes traffic from the blood to the tissues, and platelet adhesion and aggregation, maintenance of the balance between blood coagulation and fibrinolysis, control of growth, development and differentiation of the vessel wall cells) are lost or dysregulated [15]. A non-invasive method for the assessment of endothelial dysfunction is represented by flow mediated vasodilation (endothelium dependent dilation) [4].

Several authors studied endothelial dysfunction in SLE patients. The first study, performed by Lima *et al*. [10], showed that SLE patients presented lower FMD than sex and age-matched controls, even in subjects without traditional cardiovascular risk factors [10].

In our study, FMD in SLE patients was significantly lower than in control subjects ($p < 0.001$). Tani *et al.* [16] identified the same pattern of FMD in SLE patients. The reduced values of FMD in patients with SLE were found by Piper and Turner in their studies, too [13, 17].

We found a strong inverse correlation between FMD and SLEDAI ($r = -0.7321$, $p < 0.001$). This index comprises 24 items (clinical, biological, immunological): seizure, psychosis, organic brain syndrome, visual disturbance, cranial nerve disorder, lupus headache, cerebrovascular accident, vasculitis, arthritis, myositis, urinary casts, hematuria, proteinuria, pyuria, rash, alopecia, mucosal ulcers, pleurisy, pericarditis, low complement, increased DNA binding, fever, thrombocytopenia, leucopenia [8]. This correlation between FMD and the disease activity was identified in other studies [5, 10, 13]. Endothelial dysfunction is caused by several factors such as: anti dsDNA antibodies ($r = 0.7201$, $p < 0.001$), circulating immune complexes ($r = -0.4891$, $p < 0.01$), activated complement products ($r = 0.7117$, $p < 0.001$), systemic inflammation, other antibodies (antiribonucleoproteins, anti endothelial cells, antiphospholipids), total cholesterol ($r = -0.4450$, $p < 0.01$), arterial hypertension. The small number of studied patients represents a drawback in statistical analysis, but this study will continue in the future.

FMD using vascular ultrasonography on brachial artery represents a useful, non-invasive method for the assessment of the endothelial dysfunction. Reactive hyperemia produces a shear stress stimulus that induces the normal endothelium to release nitric oxide (NO), which acts as a vasodilator. Impaired endothelial function is associated with a reduced release of NO and a lower vasodilation [4].

CONCLUSION

Endothelial dysfunction is present in SLE patients even in the absence of traditional cardiovascular risk factors. It is due to disease activity. FMD using vascular ultrasonography on brachial artery represents a non-invasive, repeatable and useful method for the assessment of endothelial dysfunction.

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