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Anti-Annexin V and Cardiovascular Risk Factors in Systemic Lupus Erythematosus

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Background and Objective: Anti-annexin V has been proposed as a novel mechanism for the increased prevalence of atherosclerosis among patients with systemic lupus erythematosus (SLE). This study aimed to compare anti-annexin V levels between SLE patients and healthy controls and determine the correlation of anti-annexin with various cardiovascular risk factors.
Materials and Method: Sixty SLE patients and 30 healthy gender- and age-matched individuals were selected from outpatient rheumatology clinics in the city of Mashhad, Iran, and included in this cross-sectional study. Anti-annexin V and other parameters (including cardiovascular risk factors such as age, smoking, blood pressure, diabetes mellitus, lipoproteins levels, previous history of documented or clinically suspected atherosclerosis, obesity, premature ovarian failure, high sensitive C-reactive protein, vascular cell adhesion protein 1 and homocysteine) were measured using enzyme-linked immuno-assay.
Results: Anti-annexin V had a significant positive correlation with LDL levels ($p = 0.03$, $r = 0.28$). Anti-annexin V levels were not significantly different between the two groups ($p = 0.40$).
Conclusion: The role of anti-annexin V as a predictor of atherosclerosis risk in the lupus patients under study herein could not be confirmed.

Keywords: Annexin V, Cardiovascular Diseases, Risk Factors, Systemic Lupus Erythematosus

Introduction

Systemic Lupus Erythematosus (SLE) is an autoimmune connective tissue disease with involvement of various organs in the body. In SLE, the immune system attacks cells and tissues of the body, resulting in tissue damage and inflammation [1, 2]. Both types II and III hypersensitivity reactions are involved in the pathogenesis of this disease [3, 4]. SLE most often harms the joints, blood vessels, kidneys, nervous system, heart, skin, liver, and lungs [5, 6]. The incidence of SLE is about 9 times higher in females. Moreover, it is more common in ages between 15-35 years [4, 7]. SLE can be a fatal disease; the main cause of mortality in the long-term is cardiovascular disease related to atherosclerosis [8-10]. With recent developments in knowledge about the pathophysiology of SLE and improved diagnosis and treatment methods, life expectancy and quality of life in SLE patients have improved [11]. On the other side of the coin, due to increased life span and overall improvement in the prognosis of SLE patients, new complications have emerged [12]. Cardiovascular complications are among risk factors that influence morbidity and mortality rates in patients with SLE. It has become clear that its incidence is about 2.6-20% compared with gender- and age-matched persons without SLE [7, 13-15].

Annexin V is a ubiquitous protein with 320 amino acids, a weight of 36 KDa, and its relevant gene is located on 4q26-q28 with 13 exons and 12 introns [16, 17]. Annexin V has wide tissue distribution, including the striated muscles of the heart, vascular endothelium, chondrocytes, placenta, osteoblast, hepatocytes, and so on [18-20]. It plays a key role in the regulation of apoptosis by binding to the phosphatidylserine-exposing apoptotic cells and impeding their pro-coagulant and proinflammatory actions. It exerts anticoagulant activity as an inhibitor of the prothrombin activation process [21, 22].

Antibodies (Abs) against annexin V have been addressed in patients with SLE [23]. It has been proposed that the increased apoptosis mechanism leads to antigenic stimulation by extracellular/membrane exposition to the immune system, leading to the production of autoantibodies [24]. These Abs can interfere with functional sites of annexin V and also make immune complex, leading to a lower half-life of annexin V in plasma. These Abs are connected to the recurrent abortions and thrombotic episodes in SLE [25-27]. Anti-an-

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nexin V antibodies also inhibit the binding of annexin V to endothelium, thus inducing atherothrombosis.

Results regarding the influence of anti-annexin V antibodies on thromboembolic events in SLE, in addition to other pro-thrombotic factors, are controversial [28]. The current study evaluated possible relations between anti-annexin V antibody and some cardiovascular disease risk factors in patients with SLE and controls.

Materials and Methods

This cross-sectional study was conducted on 90 individuals, 60 of whom were lupus patients and formed the patient group and 30 individuals who formed the control group. Patients in the case group were selected from outpatient rheumatology clinics at Ghaem University hospitals in the city of Mashhad. The lupus group fulfilled the diagnostic criteria of the American College of Rheumatology [29] with a disease duration of at least one year. Individuals who did not fulfill the ACR criteria for SLE diagnosis and those who had another connective tissue disease, renal failure, recent infection, or malignancy were excluded from the study. The control group included 30 persons who matched by age and gender with the case group and were selected from individuals referring to our clinics for mechanical and non-specific pain. Informed consent was obtained from all participating patients and healthy controls.

The cardiovascular risk factors taken into account in this study comprised being older than 55 years of age, recent smoking (in the recent 10 years), blood pressure greater than 140/90 or the use of antihypertensive medication, diabetes mellitus, high density lipoprotein (HDL) levels (lower than 40 mg/dl for men and 50 mg/dl for women), low density lipoprotein (LDL) levels (higher than 160 mg/dl), previous history of documented or clinically suspected atherosclerotic disease (i.e. myocardial infarction, angina pectoris, coronary artery bypass graft, angioplasty, or stroke), peripheral artery disease, claudication and evidence of premature atherosclerosis (increased intima media thickness [IMT]) in ultrasound imaging of the carotid artery), obesity (Body Mass Index greater than 30), premature ovarian failure (younger than 45 years), high sensitive C-reactive protein (hs-CRP), vascular cell adhesion protein 1 (VCAM1) and homocysteine. Detection of antibodies against annexin was done using enzyme-linked immuno-sorbent assay (ELISA) kit (Bender MedSystem, Vienna, Austria) with adherence to the manufacturer's instruction.

The American College of Rheumatology's SLEDAI-2000 form was completed for each patient and their scores were calculated pursuant to its mandate. Further information about history of cardiac problems and risk factors was obtained using a questionnaire, physical examination, medical records, and if necessary, electrocardiography or echocardiography was carried out. Doppler ultrasound imaging of left and right common carotid was performed, determining the presence of plaque and intima media thickness (IMT).

Information from individuals in the control group about history of cardiac problems and risk factors was obtained using a questionnaire, physical examination, evaluation of possible health records in our clinic, and in some cases, electrocardiography or echocardiography was carried out. Carotid artery ultrasound imaging was also performed.

Statistical Analysis

The Kolmogorov-Smirnov test was used to assess the normality of data. For data with normal distribution, the student's t-test was applied. Descriptive analysis was shown as mean ± standard. Raw data was analyzed using SPSS version 11.5 (Chicago, IL, USA). Correlations between variables were examined using Pearson's correlation coefficient. The Mann-Whitney U test was used to assess non-normal data. A p-value of 0.05 was considered statistically significant.

This study conformed to the principles outlined in the Declaration of Helsinki. The Organizational Ethics Committee of Mashhad University of Medical Sciences also approved this study (No. 6662).

Results

The average participant age was 32.06 ± 8.90 (SD) years for the patient group and 34.19 ± 6.46 (SD) years for the control group. Using the Kolmogorov-Smirnov assay, it was determined that the distribution of age in both groups was normal. The independent samples t-test showed that the two groups were matched for age ($p = 0.062$). There were 5 males in the patient group and 4 males in the control group. The chi-square assay showed that the two groups were matched for gender ($p = 0.47$). Disease activity was assessed using ACR's SLEDAI-2000 score. The patient group had a minimum SLEDAI of zero and a maximum of thirty-one. Active disease was defined as SLEDAI > 4, while very active disease was defined as SLEDAI > 10 [4]. Among the patients, 30 (50%) had inactive disease, 14 (22.3%) had active disease, and 16 (24.7%) had very active disease.

The mean value of anti-annexin V in the patient group was 0.1661 ± 0.55 ng/ml, with a minimum of 0 and a maximum of 3.7 ng/ml. The mean value for anti-annexin V in the control group was 0.45 ± 1.5 ng/ml, with a minimum of 0 and a maximum of 7.8 ng/ml. The Mann-Whitney assay showed no significant difference between the two groups ($p = 0.93$).

A significant positive correlation was found between LDL and anti-annexin V levels ($p = 0.03$, $r = 0.28$). LDL levels were significantly different between the patient and control groups ($p < 0.001$), but anti-annexin V levels were not significantly different between the two groups ($p = 0.40$). Subjects with a history of cardiovascular complications or

evidence of atherosclerosis in carotid ultrasound were so small in number (5 cases in each category) as to make analysis meaningless. However, the levels of the anti-annexin V and LDL, (as well-established risk factors for atherosclerosis) were determined to be significant in the patient group. Anti-annexin V levels were not significantly correlated with any other evaluated risk factor in either the patient or the control group (Table 1). Other variables were taken into account in this study, including history of CVA, peripheral artery disease, history of angioplasty, history of angina pectoris or MI, history of premature ovarian failure, smoking, diabetes mellitus, presence of anti-phospholipid, and gender. However, the number of subjects positive for each of these variables was so small as to make statistical analysis meaningless.

It must be noted that among the 5 patients who had atherosclerotic plaque in the carotid, there was one case of high anti-annexin V (0.6 ng/ml). Furthermore, among the 5 patients who were positive for anti-phospholipid antibodies, there was one case with high anti-annexin V (1.5 ng/ml).

Discussion

Annexins are a heterogenous group of twelve proteins which are involved in various cell processes, including growth and apoptosis. Autoantibodies directed to several forms of annexin (including annexin V) have been reported, but their function and clinical relevance are controversial. Anti-annexin V antibodies were found in patients with autoimmune rheumatic diseases such as SLE, primary antiphospholipid syndrome, or systemic sclerosis who present with arterial or venous thrombosis. Annexin V have a high affinity for phospholipids that play an essential role in the regulation of coagulation. Anti-annexin V antibody also inhibit the binding of annexin V to endothelium, which may induce atherothrombosis. There are controversial results suggesting that anti-annexin V antibodies could be associated with thromboembolic and atherothrombotic events in lupus patients [28].

As stated in the results section, the current study could not confirm the role of anti-annexin V as a predictor of atherosclerosis risk in patients with lupus.

Despite a marked difference between the two groups re-

Table 1. The Spearman's correlation of studied risk factors with anti-annexin V levels in the Lupus group and controls.

	p-value	
Markers	**Lupus group r_s(p-value)**	**control group r_s(p-value)**
VCAM1	-0.039 (0.769)	-0.122 (0.536)
Age	-0.053 (0.690)	0.074 (0.712)
BMI	0.162 (0.221)	0.096 (0.626)
SLE duration	0.182 (0.167)	-
Right Superior IMT	0.020 (0.883)	-0.079 (0.696)
Right Inferior IMT	0.106 (0.424)	0.127 (0.526)
Left Superior IMT	-0.146 (0.269)	0.040 (0.848)
Left Inferior IMT	0.055 (0.677)	0.020 (0.925)
hs-CRP	0.030 (0.819)	-0.281 (0.147)
homocystein	-0.005 (0.969)	-0.197 (0.316)
Fasting Blood Sugar	-0.015 (0.913)	-0.089 (0.651)
Triglyceride	-0.089 (0.651)	-0.267 (0.169)
Total Cholesterol	0.097 (0.466)	0.46 (0.817)
HDL	0.104 (0.432)	0.233 (0.233)
LDL	0.089 (0.505)	0.054 (0.785)

IMT: intima media thickness, VCAM1: vascular cell adhesion protein 1, BMI: body mass index, SLE: systemic lupus erythematosus, hs-CRP: high sensivity C-reactive protein, HDL: high density lipoprotein, rs: Spearman's correlation.

garding cardiovascular risk factors, anti-annexin V levels were not significantly different.

Nor were they significantly correlated with any traditional or novel cardiovascular risk factors studied in either group, except LDL cholesterol.

There was a significant positive correlation between LDL and anti-annexin V levels that is comparable with the results of a survey by van Tits LJ et al. [30], who demonstrated that, unlike oxidized LDL (oxLDL), annexin V levels dropped as carotid stenosis progressed. Their control group had the lowest oxLDL and highest annexin V levels. They proposed that the oxLDL-to-annexin ratio can be regarded as a new and effective marker for the presence and severity of atherosclerotic carotid stenosis.

Cederholm et al. showed that lupus patients with a positive history of cardiovascular disease showed a decrease in the junction of annexin V to the surface of endothelial cells. This connection was mediated by autoantibodies among anti-cardiolipin IgG. There was also a positive correlation between annexin V attachment and intima media thickness (IMT) in their patients. The authors proposed reducing the connection of annexin V to endothelium as a new system for atherothrombosis in patients with SLE [26]. Roldan et al. further showed that plasma levels of anti-annexin V were significantly lower in young patients suffering from myocardial infarction, which can indicate a hypercoagulability state independent from the existence of anti-phospholipid antibodies [27]. In their survey, Shojaie et al. demonstrated that low annexin V levels and positive anti-annexin V is correlated to an increased tendency for thrombosis not accounted for by traditional cardiovascular risk factors [25]. Pompilian et al. found that serum annexin A5 levels in the SLE group were independent predictors for IMT and endothelial dysfunction [31]. Anti-annexin V levels were not significantly correlated with intima media thickness (IMT) in the patients under survey in the current study.

Certainly, increased risk of vascular outcomes in patients with SLE may be multifactorial and may not completely mimic traditional cerebrovascular and cardiovascular episodes. Risk factors such as hyperglycemia, hypercholesterolemia and hypertension due to renal involvement or treatment with corticosteroids, endothelial damage due to immune-complexes, thrombosis due to anti-phospholipid antibodies, vasculitis, and Libman-Sacks endocarditis may be among casual factors that play a role in cardiovascular events in SLE patients. One limitation of the current study was the exclusion of patients with renal involvement.

Conclusion

Considering the results of the present study, the role of anti-annexin V as a potential predictor for atherosclerosis risk in lupus patients remains ambiguous. Studies with larger patient groups and meta-analyses may aid in confirming the issue.

Addendum

Z. Rezaieyazdi participated in study design, patient referral, interpretation of data, intellectual content revision, and approval of the final version of the manuscript.

F. Hajizadeh-Saffar wrote the primary draft of the manuscript and participated in following up the procedure and data analysis.

S. Sedighi assisted in referring patients and gathering data.

H. Mansouritorghabeh participated in laboratory analysis of anti-annexin V and in writing the manuscript.

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Conflict of Interests

Authors affirm they have no conflicts of interest with regard to the current manuscript.

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