The Prevalence of Anticardiolipin Antibody in Patients with Systemic Lupus Erythematosus and Its Association with Clinical Manifestations

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Abstract- The central immunological disturbance in systemic lupus erythematosus (SLE) is autoantibody production. Some of these antibodies affecting components of the cell nucleus are the major characteristics of SLE. The present study was aimed to assess importance of anticardiolipin (ACL) antibody and its association with clinical state in SLE patients. A cross sectional study was performed on 100 patients with SLE referred to rheumatology outpatient clinic in Ekbatan hospital in Hamadan (Iran) between 2007 and 2008. Serum samples were extracted and screened for IgG and IgM using an ACL enzyme-linked immunosorbent assay. Up to 36% of patients were positive for ACL antibody that was more frequent in women than men (39.8% *versus* 8.3%). No association was revealed between ACL antibody and age. Clinical manifestations of antiphospholipid antibody syndrome were observed in 23.0% of patients that was more prevalent in ACL positive group compared with ACL negative group (41.7% *versus* 125%). The prevalence of other manifestations including pregnancy-related disorders (recurrent abortion), central nervous system defects, and deep vein thrombosis was 33.3%, 25.0%, and 30.6% in ACL positive group and was 9.4%, 7.8%, and 7.8% in ACL negative group that all were more frequent in the former group. The prevalence of thrombocytopenia was also higher in ACL positive group than another group (22.2% *versus* 15.6%). Among ACL positive patients with clinical manifestations of antiphospholipid antibody syndrome, 86.6% had medium to high titer of ACL. Our study emphasized value of (ACL) antibody to assess clinical status in SLE patients.

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Introduction

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease which is associated with formation of a variety of autoantibodies. Antiphospholipid antibody (APL) is an autoantibody that is linked to co-morbidities such as recurrent arterial and venous thrombosis. The increased level of APL occurs primarily or secondary to SLE or other autoimmune disorders (1,2). In the presence of this antibody, the risk of clotting can be significantly increased that its elevated level is shown to be associated with thrombotic symptoms of antiphospholipid syndrome (APS) and SLE (3). In a prospective study on 500 patients with SLE, recurrent venous thrombosis, thrombocytopenia, hemolytic anemia, recurrent fetal loss, and leg ulcers were strongly associated with the level of anticardiolipin antibody (ACL) (4).

According to the Sapporo criteria, APS can potentially be detected in subjects with clinical criteria of arterial, venous or small-vessel thrombosis as well as in those with pregnancy-related morbidities including recurrent fetal loss before the 10th week of gestation, unexplained fetal death at or beyond the 10th week of gestation, or premature birth due to placental insufficiency, eclampsia or preeclampsia (5,6).

The immunoglobulin isotype of ACL include IgG, IgM or IgA that can be detected using enzyme-linked immunosorbent assays (ELISA). The IgG isotype is most strongly associated with thrombosis (7). ACL antibodies are reported as a titer specific to the antibody isotypes (IgG, IgM, or IgA phospholipid antibody titer),

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but because of the limited accuracy and reliability of assays, consensus guidelines recommend semi-quantitative reporting of results as low, medium, or high titer (8).

APL has been also shown to bind some antigens such as prothrombin, annexin V, and β_2-glycoprotein I (β_2GPI). Antibodies reacting with these antigens may be directly involved in the pathogenesis of APL-associated symptoms (9,10). Exact mechanism of coagulopathies in presence of these autoantibodies is still unknown. Not only APL can directly bind to platelet surfaces and promote thrombo-agglutination *in vitro* (11), but it can also affect the vascular endothelium and cause prothrombotic events (12,13).

In Chinese patients with SLE, both IgG and IgM isotypes of ACL and anti-β_2GPI have been detected with ELISA kit. In a study on those patients, the highest predictive accuracy of thrombosis was with the presence of a low or higher titer of either ACL (>12 RU/ml) or anti-β_2GPI (>20 RU/ml). Also, in patients with SLE especially in those with other risk factors for thrombosis and those who treated with glucocorticoids, a transient low or high titer of ACL or anti- β_2GPI antibody had a good predictive value for the diagnosis of thrombosis (14,15). In these thrombotic events, long-term anticoagulation therapy is a choice protocol. The patients suffering from SLE and APS should be managed with long term anticoagulation, and a target international normalized ratio (INR) of 3 is recommended for their follow-up. New clotting event in patients treated to this target of INR can be decreased compared to patients treated with lower INR (15).

In the present study, we first evaluated the prevalence of positive ACL antibody in SLE patients and then assessed its association with clinical manifestations of SLE.

Materials and Methods

One hundred SLE patients (88 women and 12 men) referred to rheumatology outpatient clinic at Ekbatan hospital in Hamadan city were recruited for this cross-sectional study. All patients fulfilled the 1997 revised American College of Rheumatolog (ACR) criteria for SLE. All participants were subjected to full history, complete clinical examination, routine laboratory investigations, and IgG and IgM ACL test. Blood samples were taken at the time of patients' attendance.

ELISA were used to assess ACL level that scored as negative (<10 units) or positive (>10 units) for final analysis. ACL IgG was also classified as high (>80 unit/ml), medium (20-80 unit/ml), and low (10-20 unit/ml). Sensitivity and specificity of the test was estimated as 91.7% and 97.6%, respectively. Written informed consent was obtained from all. The data containing clinical manifestation of APS and APL antibody levels were collected in study checklist and were analyzed statistically by using SPSS software (version 15.0) along with Chi-square test or Fisher's exact test.

Results

The mean age of participants was 42 years (ranged 11-50 years) and 88% were female. Up to 36% of patients with SLE were positive for ACL antibody (39.8% of women and 8.3% of men, $P<0.05$). In the subgroups of patients with positive and negative ACL, no difference was found in term of mean age ($P=0.860$). Clinical manifestations of APS were detected in 23.0% of total patients that was significantly more prevalent in those with positive ACL antibody compared to negative ACL antibody group (41.7% *versus* 12.5%, $P<0.05$) (Table 1). Besides, clinical manifestations of APS were not seen in 87.5% of SLE patients with negative ACL test. In SLE patients with manifestations of APS, ACL test was positive in 65.0% and was negative in 35.0%. While, in SLE patients without clinical manifestations of APS, ACL test was negative in 72.7%. In 29.0% of patients with SLE, APS manifestations and results of ACL test were inconsistent. There were significant differences in overall prevalence of pregnancy-related complications such as recurrent abortion, central nervous system disorders and deep vein thrombosis in SLE patients with and without positive ACL test (Table 2). The prevalence of other manifestations including pregnancy-related disorders (recurrent abortion), central nervous system defects, and deep vein thrombosis was 33.3%, 25.0%, and 30.6% in ACL positive group and was 9.4%, 7.8%, and 7.8% in ACL negative group that all were more frequent in the former group. The prevalence of thrombocytopenia was similar in ACL positive group than another group (22.2% *versus* 15.6%). The prevalence of cardiovascular disorders, skin lesions, and arterial thrombosis in SLE patients with positive ACL test was 8.3%, 5.6%, and 5.6% and in SLE patients with negative ACL test was 6.3%, 0.0%, and 1.6% with no significant discrepancy.

Table 1. Clinical manifestations of APS in SLE patients with positive and negative ACL test.

ACL test	With clinical manifestation of APS	With clinical manifestation of APS	*P*-value
Positive	15 (41.7)	21 (58.3)	
Negative	8 (12.5)	56 (87.5)	
Total	23 (23)	77 (77)	0.001

Data are presented as number (%)

Table 2. Different types of clinical manifestations of APS in SLE patients with positive and negative ACL test.

ACL test	Pregnancy disorders	Venous thrombosis	CNS disorders	Cardiac disorders	Skin disorders	Arterial thrombosis
Test (+)	12 (33.3)	11 (30.6)	9 (25.0)	3 (8.3)	2 (5.6)	2 (5.6)
Test (-)	6 (9.4)	5 (7.8)	5 (7.8)	4 (6.3)	0 (0.0)	1 (1.6)
P-value	0.003	0.003	0.017	0.690	0.120	0.290

Data are presented as number (%)

In patients with SLE and positive ACL, the titer level of ACL was low in 50.0%, medium in 19.4%, and high in 30.6%. There was a significant difference in ACL titer level between the groups with positive and negative APS test. In SLE patients with clinical manifestations of APS and positive for ACL, IgG ACL was scored as medium to high titer in 86.6% compared with 23.8% in SLE patients with ACL positive test, but without clinical manifestations of APS.

Discussion

Our study shows that the incidence of clinical manifestations of APS and its related complications such as pregnancy-related disorders (recurrent abortion), central nervous system defects, and deep vein thrombosis in SLE patients with negative ACL test was significantly lower than those with positive ACL test. The previous studies similarly showed that the risk of thrombotic events was increased in positive APL test and has concluded that this autoantibody has an important role in the pathogenesis of APS (16,17). Up to 5% to 70% of SLE patients produce some types of ACL (18,19). In an analysis on 29 published series including more than 1000 patients with SLE, the average range of for the lupus anticoagulant was 34% and for ACL was 44%. The significant association was also found between the presence of either antibody and a history of thrombosis, neurological disorders, or thrombocytopenia (20). In another study, 37 patients with SLE were screened for APLAS (ACL antibody, lupus anticoagulant, or both) during a 27 months follow-up. In that study, 27% had evidences of APLAS and 24% had positive antibody but were asymptomatic. Therefore routine screening for ACL antibody for all patients with SLE is strongly recommended because of high risk for fetal loss and significant mortality and morbidity (21).

In the present study, no significant difference was found in mean age between the two groups of ACL positive and ACL negative patients. The age did not have an important effect on the results of ACL positive and negative test. In some studies, detected IgG antibodies against cardiolipin was reported in 30% to 40% of patients, in our study, these antibodies were similarly detected in 36% of patients with SLE (22,23).

In a general obstetric population, the prevalence of positive ACL test was similar to non-pregnant patients (24,25). In some studies, the prevalence rate in these patients ranged 5 to 70%, compared with up to 4% in healthy controls (26-29) that was consistent with our survey.

However, in our study, clinical manifestations of APS in SLE patients were reported lower than those in other similar studies (23% in comparison with 30%) (22,23). In our study, the ratio of ACL positive and negative SLE patients with clinical manifestations of APS, was 41.7% (30-50% in similar studies) and 12.5% (12-18% in similar studies) of SLE patients.

In a similar study by Finazzi *et al.,* previous history of thrombosis and the presence of an IgG ACL titer > 40 u/ml were identified as the risk factors for thrombotic events. The antibodies to phospholipids were also

detected in 50% of patients with SLE. In addition 40% of SLE patients with antiphospholipid antibody suffered thrombosis compared with 12 to 18% of SLE patients without antiphospholipid antibody. Annual stroke recurrence rate was seen in 10 of them. Up to 30% of patients with primary APS had cardiac valve thickening or vegetative lesions. Recurrent spontaneous fetal loss commonly observable in the second or third trimester was detected in 15% to 75% of positive antiphospholipid antibody women. The most common cutaneous finding was *livedo reticularis* observed in 80% of studied patients (30). In a prospective longitudinal cohort study on 1357 SLE patients, high incidence of arterial thrombosis (17.4%), venous thrombosis (25.7%), and *livedo reticularis* (31.4%) was observed in patients with ACL positive test (31). In another study, 370 APS patients were evaluated retrospectively that in 33.9% of them, APS was associated with SLE. Most patients had primary APS (n=173, 56.1%). Thrombocytopenia was seen in 29.3% that was significantly in SLE ones with APS compared to primary APS (41.9% *versus* 23.1%). Significant associations were also shown between thrombocytopenia and the appearance of other adverse events including cardiac valves thickening, cardiac dysfunction, arthritis, *livedo reticularis*, and skin ulcerations. In contrast, the rates of thrombotic episodes as well as obstetric complications were similar in patients with and without thrombocytopenia (32).

Lupus patients with ACL have an increased risk of developing thrombocytopenia ranged 11% to 40% in comparison with 4% to 17% in negative ACL subjects or in those with hemolytic anemia with the range of 2% to 8%. The symptoms traditionally thought as being associated with APS rather than SLE, such as arterial or venous thrombosis and recurrent fetal loss (16,27,29,33).

In our study, there was no significant difference in the rate of thrombocytopenia between two groups of SLE patients with positive and negative ACL test. In our study, ACL positive SLE patients with clinical manifestations of APS, medium to high titer of ACL was detected in 86.6% of cases. Similar reports were found in other previous studies regarding medium and high titer of ACL in patients with clinical manifestation of APS (23,34,35). It was also shown that the higher titer of the ACL had a high positive predictive value for diagnosis of thrombosis (23,34-36).

The majority of SLE patients with clinical manifestations of APS who had positive ACL blood tests were female in reproductive age. Blood tests for ACL can be positive in all stages of SLE, not only in progressive SLE and older ages, but also in the early stages of the disease. There is a significant difference in clinical manifestations of APS between the patients with and without positive ACL test. The positive blood tests for ACL can be a valuable marker for predicting future onset of clinical manifestations in APS and its related complications.

Asymptomatic ACL positive patients with no clinical manifestations of APS also require more attention in contrast to SLE patients with ACL negative test. In the former group, the risk factors for thrombotic events should be reduced by controlling the blood pressure, avoidance of smoking, adopting a healthy lifestyle, maintaining ideal weight and gaining safe blood cholesterol levels. Furthermore, in patients with high titer of ACL, prophylaxis with anti-platelet agents such as aspirin and avoidance of oral contraceptive pills are recommended (23,36). It appears that patients with clinical manifestations of APS who had experienced a thrombotic event (such as stroke, pulmonary emboli, pregnancy disorder, placenta insufficiency and abortion) may need treatment regardless of their plasma ACL level (15,23,36).

Despite the presence of negative ACL patients with clinical manifestations and positive ACL cases without clinical manifestations, blood tests for ACL are recommended as a screening test. All the positive ACL patients with SLE need to be closely followed up and the risk factors for thrombotic events and atherosclerosis should be reduced. Negative ACL patients with clinical manifestations of APS may turn into positive ACL state. On the other hand, positive ACL patients without clinical manifestations of APS may develop the clinical manifestations of APS. In conclusion, our study emphasized the value of ACL antibody to assess clinical status in SLE patients. Larger cohort studies are recommended to determine the time of prospective seroconversion of ACL and appearance of the clinical manifestations of APS in the SLE patients.

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