Prevalence of Antiphospholipid Antibodies in Sample of Iraqi Patients with Systemic Lupus Erythematosus: A Cross Sectional Study

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Abstract **Objective:** Antiphospholipid antibodies (APLAs) have high risk of vascularthrombosis with significant clinical comorbidities. Anticardiolipin antibodies (ACLAs)and Lupus anticoagulant (LA) are important APLAs. The aim of this study was to evaluate the prevalence of APLAs(ACLAs and LA) and their clinical significance among sample of Iraqi patients with systemic lupus erythematosus patients (SLE). **Patients and methods:** A single center cross sectional study conducted on 50 SLE patients diagnosed according to the 1997 revised American College of Rheumatology (ACR) criteria for SLE from February 2010 to April 2011. Patients' age at SLE diagnosis, disease duration, SLE disease activity index (SLEDAI), renal involvement, cerebral involvement, cardiac involvement, pregnancy events, and thrombotic events were analyzed. Serum samples were extracted and screened for IgG and IgM using an anticardiolipin (ACL) enzyme-linked immunosorbent assay, Lupus anticoagulant (LA), prothrombin time (PT), partial thromboplastic time (PTT), kaolin clotting time (KCT), and KCT index were assessed in all patients. **Results:** Of 50 SLE patients, the prevalence of positive anticardiolipin antibodies (ACLA) was 10(20%) and positive LA 5 (10%). Abnormal KCT12 (24.5%), Abnormal KCT index 5(10), Abnormal PTT2 (4.1%), and Abnormal PT 2(4%). Thrombotic events, pregnancy events, and cerebral involvement were associated with positive serology (P = 0.000, 0.225, 0.083 respectively). Renal and cardiac involvement were associated with negative serology (P = 0.019, 0.094 respectively). No new thrombotic events were found. **Conclusions:** Prevalence of positive ACLAs was 20% and positive LA 10%. Thrombotic events, pregnancy events, and cerebral involvement were associated with positive serology while renal and cardiac involvement with negative serology. We suggest screening SLE patients for the presence of APLAsand larger sample with longer follow up for their clinical manifestations.

Keywords: antiphospholipid antibodies, systemic lupus erythematosus, anticardiolipin antibodies, autoantibodies and SLE

1. Introduction

Systemic lupus erythematosus (SLE) is a multisystem autoimmune connective disease which is associated with formation of a variety of autoantibodies [1]. Antiphospholipid antibodies (APLAs) are a heterogeneous group of immunoglobulins that target membrane phospholipids or phospholipid-protein complexes involved in the occurrence of thrombotic events and recurrent pregnancy loss in patients with antiphospholipid syndrome(APS) [2]. APLAs appear mainly in association with connective tissue diseases such as SLE. In fact, they form part of the diagnostic criteria for SLE [3]. But they are also found in other situations [4]. Various infectious agents, tumors, and drugs can cause APLAs to appear transiently, usually without the appearance of anti-β2Glycoprotein I (anti-

β2GPI) and only rarely in association with thrombotic tendency [5,6].

The exact mechanism of coagulopathies in presence of these autoantibodies is still unknown. Not only APLAs can directly bind to platelet surfaces and promote thrombo-agglutination in vitro [7], but it can also affect the vascular endothelium and cause prothrombotic events [8,9].Various studies have investigated the presence of APLAs in patients with SLE and reported prevalence figures ranging from 24% to 60%. [10,11]. In Chinese patients with SLE, both IgG and IgMisotypes of ACLAs and anti-β2GPI 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-β2GPI (> 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 ACLAs or

anti- β2GPI antibody had a good predictive value for the diagnosis of thrombosis [12,13]. In these thrombotic events, long-term anticoagulation therapy is a choice protocol. The objectives of this study were to evaluate the prevalence of APLAs in a sample of Iraqi patients with SLE and to assess their clinical importance.

2. Patients and Methods

2.1. Study Design

This was a single center cross sectional study conducted at medical wards, rheumatology wards, and rheumatology consultation clinic at Baghdad Teaching Hospital, Medical City, Baghdad, Iraq from February 2010 till April 2011.Patients were screened for SLE and evaluated for APLAs and their clinical significance was assessed. Informed consent was obtained from all participants and this study was approved by the ethical committee of Baghdad University, College of Medicine, Medical Department.

2.2. Sample Selection

A total of 50 SLE patients were recruited in this study. Eligible patients had confirmed the 1997 revised American College of Rheumatology (ACR) criteria for SLE [14]. The exclusion criteria included all patients with mixed connective tissue disease, overlap syndrome, or having other comorbid diseases.

2.3. Clinical Evaluation

All participants were subjected to full history and complete clinical examination. Patient's age, sex, duration of SLE, age of patient at SLE diagnosis, and SLE disease activity measured by SLE disease activity index (SLE DAI) [15].

History of drug intake that can give false positive antiphospholipid antibody test as prednisolone, immunomodulators, non-steroidal anti-inflammatory drugs(NSAIDs), and hydroxychloroquine. History of nephritis, thrombotic events and the number of these events, pregnancy events (unexplained deaths, abortions, and premature birth) and history of predisposing factor at time of thrombosis (bed rest, surgery, and drugs).All patients were followed up for at least 6 months for any evidence of new thrombotic events at the rheumatology clinic or by patient's calling. The patients had been tested for each of the APLAs (ACLAs and LA) on 2 occasions at least 12 weeks apart, and those with a positive result in both tests were included in the study.

2.4. Laboratory Evaluation

Blood samples were taken at the time of patients' attendance. ELISA technique (EUROIMMUN Medizinische Labordiagnostike, Germany) was used to assess anticardiolip in antibodies (ACLAs)(IgG and IgM) (negative < 12 RU/ml). LA was detected by the prolongation of activated partial thrombo plast in time (aPTT) in a mixing test, using the 1/5 diluted aPTT reagent (Automated aPTT, General Diagnostics, USA).Complete blood count, prothrombin time (PT) (control 13-13.5 sec), partial thromboplastin time (PTT) (control 28-40 sec), kaolin clotting time (KCT) (control 60-100 sec), KCT index (normal value < 1.2), and Urine analysis were also measured.

2.5. Appropriate Other Investigations

Direct Coomb's test, correction study (for PT, PTT, and KCT), ECG, CXR, echocardiography, abdominal ultrasound, CT scan of brain, bone marrow study, and doppler study were done when indicated.

2.6. Statistical Analysis

Statistical package for social sciences version 18 (SPSS 18) was used for data input and analysis. Discrete variables were presented as numbers and percentages and Continuous variables as mean and standard deviation (SD). Chi square test for goodness of fit was used to test the significance of observed distributions. Chi square test for independence and Fisher's exact test where appropriate were used to test the significance of association between discrete variables. In places where cells have small expected values and chi square does not operate optimally; condensation for rows was done. Student t- test or Mann-Whitney test where appropriatewere used to test the significance of difference between two means. All tests were two sided and used asymptotic P value. Findings with P value less than 0.05 considered significant.

3. Results

Of a total 50 SLE patients involved in the study, there were 49(98%) females and 1(2%) male with their mean ages 31.9 ± 9.8 years and range 14-55 years. Positive ACLAs were observed in 10(20%) patients, Positive LA5(10%), Abnormal KCT12 (24.5%), Abnormal KCT index5(10%), Abnormal PT (> 15.5 sec) 2 (4%) Abnormal PTT (> 48 sec) 2 (4.1%) as shown in Table 1.

Table 1. Baseline demographic, clinical, and laboratory characteristics of SLE patients (n = 50)

Variables	Value
Age (year); Mean ± SD	31.9 ± 9.8
Range	14 - 55
Gender; n (%)	
Male	1(2.0 %)
Female	49(98.0%)
Renal Manifestations n (%)	18(36%)
Hematological manifestationsn (%)	29(58%)
Neurologic manifestationsn (%)	6(12%)
Thrombotic eventsn (%)	10 (20%)
Pregnancy eventsn (%)	9 (18%)
Active SLE on recruitmentn (%)	25(50%)
Drugs n(%)	
Prednisolonen (%)	45(90%)
Cyclophosphamiden (%)	9(18%)
Azathioprine n (%)	19(38%)
Chloroquinen (%)	20(40%)
Mycophenlatemofetiln (%)	1(2%)
NSAIDsn (%)	46(92%)
Positive ANAn (%)	49(98%)
Positive Anti-DNA Abn (%)	28(56%)
Positive Anti-SmAbn (%)	1(2%)
Positive ACLAs	10(20%)
Positive LA	5(10%)
Abnormal PT (>15.5 sec)	2(4%)
Abnormal PTT (> 48 sec)	2(4.1%)
Abnormal KCT	12 (24.5%)
Abnormal KCT index	5(10%)

SD, standard deviation; n, number, ANA, antinuclear antibody; Anti-dsDNA, anti-double stranded deoxyribonucleic acid; Ab, antibody, Anti-Sm, anti- smith**,** NSAIDs, non steroidalanti inflammatory drugs; n, number, ACLA, anticardiolipin antibodies; LA, lupus anticoagulant; PT, prothrombin time; PTT, partial thromboplastin time; KCT, kaolin clotting time.

On comparing between positive serology(n = 14) and those with negative serology (n = 36), we found thatthrombotic events were significantly more in positive serology (P = 0.0001) while renal involvement was significantly more in negative serology (P = 0.019). Additionally, Cerebral and pregnancy events were more in positive serology but statistically not significant (P = 0.083, 0.225 respectively). Also, cardiac involvement was more in negative serology (P = 0.094) (Table 2).

Table 2. Comparison between the positive serology and the negative serology groups of patients

Variables	Serology		Total(n = 50)	p
	positive (n = 14)	Negative(n = 36)		
Age at Diagnosis (year), Mean ± SD	27.8 ±10.7	28.2 ± 10.0	28.1 ±10.1	0.906
Duration of SLE year, Mean ±SD	4.6 ± 5.6	3.6 ± 5.5	3.9 ± 5.5	0.573
Active SLE on recruitment n(%)	8(57.1)	17(47.2)	25(50)	0.529
Renal involvementn(%)	3(21.4)	21(58.3)	24(48)	0.019*
Cerebral involvementn(%)	5(35.7)	5(13.9)	10(20)	0.083
Cardiac involvementn(%)	2(14.3)	14(38.9)	16(32)	0.094
Pregnancy eventsn(%)	4(28.6)	5(13.9)	9(18)	0.225
Thrombotic eventsn(%)	7(50)	3(8.3)	10(20)	0.0001**
New thrombotic eventsn(%)	0(0)	0(0)	0(0)	

*p < 0.05 significant; **P < 0.001 highly significant; SD, standard deviation

4. Discussion

Antiphospholipid antibodies (APLAs) are associated with a serious autoimmune condition termed 'antiphospholipid (antibody) syndrome' (APS) and linked to a significant clinical co-morbidities such as recurrent vascular thrombosis [16].

This study evaluated APLAs among SLE patients and their clinical significance. It showed that ACLAs were present in 20% and LA in 10%. Various studies have analyzed the presence of APLAs in patients with SLE and reported prevalence figures ranging from 24% to 60%. [10,11]. Other published studies reported that prevalence of ACLAs ranged from 17% to 86% [17,18] and LA ranged from 11-30 % [18]. One of the more recent studies, published by Petri [19] in 2010, found that 47%ofpatients were positive for ACLAs, and 26% for LA.

In addition, thrombotic events were significantly associated with positive serology. This finding was similar to a study performed at Dong-A university college of medicine in Korea where 88 SLE patients were studied and tested for APLAs. Positivity for LA was significantly associated with venous/arterial thrombosis whereas positivity for IgG and IgM ACLAs was not significantly associated with thrombotic events [20]. Another study done in Helsinki where two groups of SLE patients were followed for thrombotic events, the group with positive LA reported highly significant more attacks of deep vein thrombosis in comparison with the negative group [21].

Another observation of note, pregnancy events had an association with positive serology but this association was statistically not significant (P = 0.225). In the same study done in Korea, positivity for LA and ACLAs neither of them was significantly associated with pregnancy loss [20].

Women with APLAs had an unusually high proportion of pregnancy losses within the fetal period. Pregnancies in women who were positive for APLAs could also be complicated by premature delivery due to pregnancy-associated hypertensive disease and uteroplacental insufficiency [20,22]. A retrospective study done in Portugal on 136 pregnant SLE patients found significant history of fetal losses among APLAs positive group and a significantly higher non-successful outcome of pregnancies in same group in comparison with the APLAs negative SLE pregnants [23].

In the present study, renal involvement was significantly associated with negative serology. Bhandari et al found that ACLAs were a strong predictor of intra-glomerular thrombi in SLE patients with renal involvement and reported that this conferred a worse long term renal outcome [24]. In a study done in Helsinki (two groups of SLE patients one with positive LA and the other with negative LA were followed for 22 years), nephritis did not correlate with presence of LA [21].

Moreover, the current study showed that cardiac involvement had an association with negative serology but this association was not significant. Similar finding was reported by Woo et al who reported that cardiac disorders were more in seronegative ACLAs [22]. A nother study done at Hammersmith hospital in UK to determine the association between cardiac abnormalities and raised ACLAs in SLE found a strong association between myocardial, valvular involvement in SLE and raised ACLAs [25]. Also, a study done in Italy where 60 SLE patients and 30 controls were evaluated by echocardiography to detect cardiac abnormalities and to define the possible correlation with APLA reported no clear correlation was evident between endocardial or pericardial involvement and such autoantibodies. On the contrary the demonstration of APLAs in the patients with regional or global left ventricular dysfunction could suggest a pathogenic role of these autoantibodies in myocardial hypokinesis. Therefore, APLAs could represent only one of the pathogenic factors of the cardiac lesions in SLE patients, together with immunologic and iatrogenic factors. The involvements of other systems as

renal, vascular and pulmonary certainly play an important role in predisposingsecondary cardiac manifestations [26].

Furthermore, this study showed that cerebral involvement was associated with positive serology but this association was not significant. In a study done at Helsinki, Cerebral artery occlusions were significantly more common in patients with LA [21]. Also, Basiri et al reported that central nervous system defects were significantly more in ACL positive patients [17]. The small sample size may explain the non statistical significance in our study.

The main limitations of this study were: First; the small number of patients with short follow up period so the findings need to be confirmed in a larger longer prospective study. Second, we could not do antiB2GPI for ethical reasons due to unavailability. However, our study had points of strength. First, well defined inclusion criteria of SLE patients without overlapping with other connective tissue diseases or inflammatory arthritis, and comorbid diseases that may affect the results. Second, up to best of our knowledge, the current study is the first study to report APLAs in a sample of Iraqi SLE patients.

In conclusion, APLAs were relatively high in a sample of Iraqi SLE patients. Prevalence of positive ACLAs was 20% and positive LA 10%. Thrombotic events, pregnancy events, and cerebral involvement were associated with positive serology while renal and cardiac involvement with negative serology. We suggest screening SLE patients for the presence of APLAs and longer follow up for their clinical manifestations.

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