Zagazig University Medical Journal
www.zumj.journals.ekb.eg

Volume 28, Issue 1, January 2022, Page 130-136 XML

Manuscript ID ZUMJ-1911-1632
DOI 10.21608/zumj.2020.20187.1632

ORIGINAL ARTICLE

Frequency of Antiphospholipid Antibodies in Systemic Lupus Erythematosus Patients and Their Clinical Associations

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Submit Date	2020-01-11 05:02:40
Revise Date	2020-02-02 14:23:53
Accept Date	2020-02-05 12:29:35

ABSTRACT

Background: Antiphospholipid syndrome can occur as an isolated diagnosis condition or associated with other autoimmune diseases such as systemic lupus erythematosus(SLE). This study aimed to determine frequency of antiphospholipid (aPL) antibodies in SLE patients and to evaluate their relation to disease manifestations, activity index and damage index of SLE.

Patients and methods: This study included a total of 73 SLE patients. They were 63 females and 10 males, their ages ranged from 18-49 years. Full history taking, complete clinical examination, disease activity assessment by SLEDAI-2K score and organ damage assessment by SLICC/ACR damage index were done to all study patients. Human lupus anticoagulant (LA), IgM and IgG anticardiolipin antibodies(aCL) were measured by double antigen sandwich enzyme-linked immunosorbent assay (ELISA).

Results: We found that aCL-IgM (21.9%) was the most common aPL antibody in the patients of our study followed by LA (17.8%) then aCL-IgG (16.4%). There was statistically significant difference in percentage of positive antiphospholipid antibodies as regard the disease activity index (SLEDAI-2K), SLICC/ ACR damage index, venous thrombosis, stillbirth, abortion, and cerebrovascular manifestations.

Conclusion: aPL antibodies are not infrequent finding in SLE patients. There was a significant correlation between aPL antibodies and both disease activity, and degree of organ damage. Also, our results clarified the correlation between positive aPL serology and various thrombotic events and pregnancy losses in SLE patients.

Keywords: Antiphospholipid antibodies; lupus anticoagulant; anticardiolipin antibodies; systemic lupus erythematosus

INTRODUCTION

Systemic lupus erythematosus (SLE) is a disease of young women in their childbearing years, with the potential to cause significant physical disfigurement, morbidity, and, occasionally, mortality. The hallmark of SLE, is the presence of autoantibodies, such as anti-phospholipid (aPL) antibodies, deposition of immune complexes in tissues and excessive complement activation**[1].** High levels of anti-phospholipid (aPL) antibodies are commonly associated with the anti-phospholipid syndrome (APS) which presents with venous or arterial thrombosis and/or recurrent spontaneous abortions **[2].** These antibodies include anti-cardiolipin antibodies, lupus anticoagulant, β2 glycoprotein 1 (β2GP1) and anti prothrombin antibodies **[3].** aPL antibodies can be primary or secondary in nature. Secondary aPL can be caused by certain coexisting diseases (e.g., SLE, immune thrombocytopenic purpura, leukemia, cancers, and some infections) or by the treatment with certain drugs (eg, chlorpromazine, procainamide) **[4].** The thrombotic tendency of patients with

antiphospholipid antibodies possibly derive from adverse effects of these autoantibodies on coagulation proteins, platelet activation and endothelial cell contributions **[5].** Lupus anticoagulants (LA) are antibodies directed against plasma proteins (eg, prothrombin bound to anionic phospholipid). They block the assembly of the prothrombinase complex resulting in prolongation of in vitro clotting assays **[6].** Anticardiolipin antibodies (aCL) react with phospholipids such as cardiolipin and phosphatidylserine. The aPL antibodies can appear in different ways such as asymptomatic patients or APS with recurrent venous and/or arterial thrombosis, APS with recurrent pregnancy loss, patients with aPL positivity with other manifestations (i.e. thrombocytopenia, hemolytic anemia or livedo reticularis) **[7]** and in a small number of patients, as an abrupt, life-threatening complication due to multiple thromboses of medium and small arteries occurring over a period of days which is termed catastrophic APS **[8].** The aim of this work was to determine frequency of antiphospholipid antibodies in SLE patients and to evaluate its relation to disease manifestations, activity index and damage index of SLE.

PATIENTS AND METHODS

This study was performed in the Rheumatology and Rehabilitation department of Zagazig University Hospitals, after review and approval by the Institutional Review Board, Faculty of Medicine, Zagazig University. They were 62 females and 11 males, and their mean age (range) was 31±8.3 (19-49) years. Disease duration ranged from 1-18 years. Our patients were fullfilling Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE **[9].** Written informed consent was obtained from all participants. This study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Clinical assessment;

All patients were subjected to full history taking, clinical examination, disease activity assessment by SLEDAI-2K score **[10]** and organ damage assessment by SLICC/ACR damage index **[11].** Activity levels were defined on the basis of SLEDAI scores **[12].** No activity (SLEDAI; 0), mild activity (SLEDAI; 1–5), moderate activity (SLEDAI; 6–10), high activity (SLEDAI; 11–19), very high activity (SLEDAI ≥ 20).

Laboratory investigations;

All cases were subjected to the following laboratory tests: complete blood picture, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP),C3 and C4, liver functions, kidney functions, ANA and Anti-dsDNA antibodies titre, antiphospholipid antibodies including: lupus anticoagulant (LA), IgM and IgG anticardiolipin antibodies (aCL- IgM), (aCL-IgG). Serum Human lupus anticoagulant were measured by double antigen sandwich enzyme-linked immunosorbent assay (ELISA) according to the instruction of the manufacturer (SunRed, Shanghi). Serum Anti-Cardiolipin IgG/IgM was measured by double antigen sandwich ELISA according to the instruction of the manufacturer (ORGENTEC Diagnostika GmbH, Germany). Blood was collected by veni-puncture, serum- coagulation at room temperature 10-20 min, centrifugation 20-min at the speed of 2000-3000 r.p.m were done. & supernatant was removed. Serum was assayed immediately after storage at-20° C (without repeated freeze-thaw cycles).

Statistical analysis;

IBM SPSS statistics (V. 23.0, IBM Corp., USA, 2015) was used for data analysis. Data were expressed as Mean ±SD for quantitative parametric measures in addition to both number and percentage for categorized data. Comparison between two independent mean groups for parametric data was done by using Student t test and among more than 2 patient groups using Analysis of Variance (ANOVA). Chi-square test was used to study the association between each 2 variables or comparison between 2 independent groups as regards the categorized data. The probability of error at 0.05 was considered significant, while at 0.01 and 0.001 was considered highly significant. Regression analysis and ROC curve were used to determine the role of aPL antibodies in prediction of disease activity.

RESULTS

This study included 11 male and 62 female SLE patients. We found that aCL-IgM was the most common aPL antibody in our patients (21.9%) followed by LA (17.8%) then aCL-IgG (16.4%) (table 1, figure 1).There was statistically significant difference between positive (LA, aCL-IgM, aCL-IgG) SLE patients compared to negative patients regarding venous thrombosis. There was significant difference between SLE patient with positive and negative aCL-IgM regarding abortion ($p<0.05$). There was significant difference between SLE patient with positive and negative LA regarding stillbirth ($p<0.05$) (table 2). As regard clinical manifestations of SLE, there was statistically significant difference between positive (LA, aCL-IgM, aCL-IgG) SLE patients compared to negative patients regarding cerebrovascular manifestations only ($p<0.05$) (table 3).

There was statistically significant positive correlation between antiphospholipid antibodies, and; ESR, CRP, and anti-dsDNA **level, ($p<0.05$).** On the other hand, there is significant negative correlation between antiphospholipid antibodies and both C3 and C4 **($p<0.05$)** (table 4).

There was significant positive correlation between LA and both of serum creatinine and proteinuria but there is significant negative correlation between LA and creatinine clearance ($p<0.05$) (table 4). There was statistically significant difference between percent of positive antiphospholipid antibodies and the degree of disease activity in SLE patients ($p<0.05$) (table 5). There was statistically significant difference between percent of positive antiphospholipid antibodies and degree of tissue damage in SLE patients ($p<0.05$) (table 5). aCL-IgG was a significant predictor for disease activity in SLE patients ($p<0.05$) (table 6).

Table (1): Frequency of antiphospholipid antibodies in the SLE patients

Antiphospholipid antibodies	No (%)	Mean ±SD	Range
LA	13(17.8)	31.7±14.8	16.35-55.13
aCL -IgG	12(16.4)	20.7±10.8	10-43
aCL -IgM	16(21.9)	22.1±27	7-120

LA; lupus anticoagulant, aCL-IgG; anticardiolipin antibody -IgG,aCL-IgM: anticardiolipin antibody- IgM

Table (2): Relation between antiphospholipid antibodies (LA, aCL –IgG and aCL -IgM) and thrombotic &pregnancy manifestations of antiphospholipid syndrome

Manifestation	LA		aCL -IgG		aCL -IgM	
	+ve(13)	P	+ve(12)	P	+ve(16)	p
Thrombotic events (no=14)	12 (92)	<0.001**	6 (50)	0.008*	9 (56)	<0.001**
Abortion (no=16)	4(40)	0.26	5(41.7)	0.26	7(50)	0.03*
Stillbirth (no=5)	3 (30)	0.026*	2 (16.7)	0.24	1 (7)	0.7

*p<0.05 is statistically significant **p≤0.001 is statistically highly significant

Table (3): Relation between LA, aCL-IgG and aCL-IgM and clinical manifestations of SLE

Manifestation	LA			aCL -IgG			aCL -IgM		
	+ve(13)	-ve(60)	p	+ve(12)	-ve(61)	p	+ve(16)	-ve(57)	p
Cerebrovascular	5 (38.5)	5(8.5)	0.013*	5 (31.3)	5(8.2)	0.028*	5 (31.3)	5 (8.8)	0.035*

Manifestation	LA			aCL -IgG			aCL -IgM		
Cardiac	2(15)	6(10)	0.4	1(8)	7(11)	0.6	3(19)	5(9)	0.2
Pulmonary	3(23)	3(5)	0.06	2(16)	4(6.4)	0.25	2(12.5)	4(7)	0.6
Cutaneous	0 (0)	4 (6.6)	0.4	2(16)	2(3.2)	0.12	1 (6)	3 (5)	0.6

*p<0.05 is statistically significant

Table (4) Correlation between antiphospholipid antibodies (LA, aCL –IgG and aCL -IgM) and laboratory Findings

Laboratory finding	LA		aCL -IgG		aCL -IgM	
	r	p	r	P	r	P
ESR level (mm/hr)	0.32	0.001**	0.28	0.015*	0.26	0.02*
CRP level (mg/dl)	0.25	.027*	0.31	0.001**	0.23	0.03*
Serum creatinine (mg/dl)	0.33	0.001**	-.009	.939	.056	.638
24 hours urine protein	0.4	0.001**	.028	.814	.009	.940
Creatinine clearance	-0.28	0.015*	-.091	.442	-.069	.561
Anti ds-DNA	0.26	0.02*	.356	.001**	.187	.114
C4	-0.379	0.044*	-0.134	0.258	-0.026	.825
C3	-0.283	0.048*	-0.03	0.980	-0.104	.383

ESR; Erythrocyte sedimentation rate, CRP; C reactive protein, C; complement *p<0.05 is statistically significant **p≤0.001 is statistically highly significant

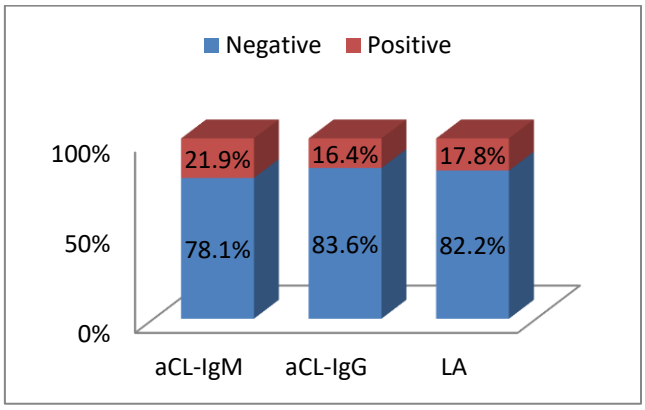
Table (5): Relation between antiphospholipid antibodies (LA, aCL -IgG and aCL -IgM) and SLEDAI score & damage index in SLE patients

Antiphospholipid antibodies	LA	aCL-IgG	aCL-IgM	P
SLEDAI score	8.2	10.5	10.6	0.01(S)
damage index	0.4	1.96	2.16	0.001(S)

SLEDAI; SLE Disease Activity Index

Table (6): Multi-linear regression illustrates for antiphospholipid antibodies (LA, aCL -IgG and aCL -IgM) as predictor factors for disease activity in SLE patients

Model	Unstandardized Coefficients		Standardized Coefficients	t	P (Sig.)
(Constant)	8.164				
LA level	.093	.097	.124	.964	.339
aCL –IgG level	.336	.131	.358	2.558	.013(S)
aCL –IgM level	-.001	.072	-.002	-.015	.988



Figure(1): Antiphospholipid antibodies (LA, aCL-IgG and aCL-IgM) in SLE patients.

DISCUSSION

Anti-phospholipid antibodies (aPLs) are a family of autoantibodies directed against phospholipid-binding plasma proteins, most commonly β2-glycoprotein 1 causing vascular thrombosis and pregnancy losses**[13].** aPL antibodies can be associated with connective tissue diseases such as SLE and form one of its classification criteria **[14].** The most common aPL antibodies are LA and aCL antibodies. The current study aimed to determine the frequency of aPL antibodies, evaluate its relation to disease manifestations, disease activity and damage index in SLE patients. We found that aCL-IgM (21.9%) was the most common aPL antibody in our patients, followed by LA, it was (17.8%), lastly aCL -IgG was (16.4 %).

These findings agreed with those of a study done by Noori et al., in 2013 who investigated 50 SLE patients and found anticardiolipin antibodies was found in 20% and LA in 10% of their studied patients **[15].**
There was statistically significant difference between percentage of positive antiphospholipid antibodies and the degree of disease activity in SLE patients. Our findings agreed with Lam and Petri who observed that anticardiolipin antibodies may be a useful marker in monitoring disease activity in patients with SLE **[16].**

Our results showed that aCL -IgG is a significant predictor for disease activity in SLE patients. This finding goes ahead with a study done by Reveille who found that aCL levels were of value in predicting disease activity and monitoring SLE patients who completely or partially fulfilled the criteria for APS**[17].**

There was a positive significant correlation between LA and serum creatinine and proteinuria but negative significant correlation between LA and creatinine clearance.
This finding agreed with a study that showed that aPL antibodies were frequent findings in SLE patients and LA tended to associate with lupus nephritis, and proteinuria and could identify patients who had increased risk to develop poor renal outcomes **[18].** Sciascia et al, observed that renal prognosis was affected by the presence of aPL in patients with lupus nephritis, that require different therapeutic approach **[19].**

There was statistically significant difference in positive LA, aCL-IgM, aCL-IgG,SLE patients compared to negative patients regarding venous thrombosis. This finding goes ahead with a study done by Somers et al who concluded that SLE patients positive for LA and aCL were at substantial risk for venous thrombosis over time **[20].** The results of another study confirmed that LA was strongly associated with venous thrombosis particularly in young patients **[21].** Moreover, Domingues et al observed that aCL-IgG and LA were associated with higher rates of thromboses in SLE patients **[22]**.

Our study revealed that aPL antibodies positivity was strongly associated with stillbirth and abortion. These results agreed with a retrospective study on 136 pregnant SLE patients that found significant history of fetal losses among aPL positive group and a

significantly higher poor outcome of pregnancies in same group in comparison with the aPL negative pregnant SLE patients **[23].**

In contrast to the previously mentioned studies, a retrospective study on 62 pregnancies in 50 SLE patients showed that adverse fetal outcome was not affected by aPL (LA test, aCL, or β2GPI) or APS diagnosis. The authors of this study explained their results as fetal outcomes in lupus patients are influenced by awareness among SLE patients, the pre-conceptual counseling, postponing pregnancy until disease remission, multidisciplinary care of SLE patients during pregnancy, advances in treatment modalities and good antenatal care **[24]**.

Moreover, there was a statistically significant difference in positive aPL patients regarding cerebrovascular manifestations. This finding agreed with a study that showed that cerebral involvement in SLE was associated with positive aPL serology **[15]**. Also**,** another study reported that central nervous system defects were significantly more in aCL positive patients **[14]**. The previous findings were clarified by Oosting et al as they demonstrated that aPLs may bind to neurons or glial cells and disrupt their function, also aPLs may interfere with endothelial cells function and promote their procoagulant activity **[25]**.

We found that aPL antibodies positivity correlated with anti ds-DNA titre, C3 and C4 levels. These results agreed with a study performed by Garabet et al, who found a significant association between the presence of aPL in patients with SLE and low complement levels of C3 and C4**[26]**.

There was statistically significant difference in percentage of positive antiphospholipid antibodies regards to degree of tissue damage.

This finding agreed with some studies that stated that a significant aPL profile was associated with an increased risk of organ damage **[27]**, **[28]**.

The relatively small sample size was the main limitation of our study.

In conclusion, this study showed that, aPL antibodies are not infrequent finding in SLE patients. There was a significant correlation between aPL antibodies and both disease activity, and degree of organ damage. Also, our results clarified the correlation between positive aPL serology and various thrombotic events and pregnancy losses in SLE patients.

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TO Cite
Abdelal, I., Gaballah, N., Kamel, B., Kamel, L. Frequency of antiphospholipid antibodies in systemic lupus erythematosus patients and their clinical associations. *Zagazig University Medical Journal*, 2022; (130-136): -. doi: 10.21608/zumj.2020.20187.1632