THE PREVALENCE OF ANTIPHOSPHOLIPID ANTIBODIES AND THEIR CORRELATION WITH CLINICAL, LABORATORY FINDINGS IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS

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ABSTRACT

Objective. *To evaluate the prevalence of antiphospholipid antibodies (aPL Abs) in systemic lupus erythematosus (SLE) patients and the relationship between these Abs with clinical and laboratory findings.* ***Methods.*** *Cross-sectional descriptive study on 60 patients newly diagnosed with SLE according to SLICC 2012 criteria, at the Center for Allergy - Clinical Immunology, Bach Mai Hospital from October 2022 to August 2023. The aPL group were defined as positive for at least one aPL. The clinical manifestation and laboratory findings of the positive and negative aPL groups were compared.* ***Results****. 37/60 patients (61.67 %) had positive for at least one aPL Abs. The percentage of groups positive for single antibody aCL, LAC, β2GPI, aPL, double and triple antibodies are 46.67%, 30%, 20%, 11.67%, 35%, 18.33%, respectively. We found that positive aPL and LA Abs significantly correlated with chronic kidney disease as compare with negative group. Unfortunately, no significance was found in hematology, neurology, thromboembolism, obstetric pathology, or livedo manifestations between the two groups.* ***Conclusion.*** *61.7% newly SLE diagnosis was positive for at least one aPL Abs and the presence of these Abs was independently associated with the risk chronic kidney disease in SLE patients.*

Keywords: *systemic lupus erythematosus, antiphospholipid syndrome, lupus anticoagulation, anti-cardiolipin antibody, anti-beta2-glycoprotein-I antibody*

1. INTRODUCTION

Systemic lupus erythematosus (SLE) is the most common systemic autoimmune diseases characterized by the presence of several autoantibodies (Abs) and organ damage related to immune complex-mediated microvascular injuries. Clinical features are variable from mild joint and skin involvement to life-threatening renal, hematologic, and/or central nervous system manifestations.[1] SLE patients have been shown to have an increased risk of blood clotting formation, venous and arterial thromboembolism, and high risk of fetus loss.[2] Several factors that have been demonstrated to increase these risks in SLE patients such as immune complex-related vascular damage, the presence of lupus anticoagulants (LA) and antiphospholipid antibodies (aPL Abs), and acquired or inherited thrombophilic defects as well as placental dysfunction.[2-4]

Antiphospholipid syndrome (APS) is mainly classified into primary and secondary; in which secondary APS is often closely related to autoimmune diseases, the most common is systemic lupus erythematosus.[5] Clinical manifestations of APS are veins, arteries, and small blood vessels thrombosis as well as obstetric events such as gestational gestation, recurrent early miscarriages, intrauterine growth restriction, or severe preeclampsia.[5,6]

aPL Abs are a heterogenous group of autoantibodies, including LA, anticardiolipin antibodies (aCL), and/or anti-beta2-glycoprotein-I antibodies (β2GPI).[7] Although up to 40% of patients with SLE have positive for at least one aPL Ab, however, less than 40% will eventually experience thrombotic events.[7] APS has been demonstrated to develop in 50%~ 70% of patients with both SLE and positive for at least one aPL Abs after 20 years of follow up. Moreover, positive aPL Abs have been associated with the risk of mortality and visceral organs damage, such as thrombocytopenia, valvular heart disease and/or neuropsychiatric manifestations.[8-10]

In Vietnam, the clinical and laboratory findings of APS in SLE were reported. aCL positive is commonly found in SLE patients.[11,12] However, the aPL antibody profiles in newly SLE diagnosis have not been shown. Therefore, we conducted this research with two goals:

1. To clarify the prevalence of aPL antibodies in newly SLE diagnosed.
2. To evaluate the relationship between aPL antibody profiling with clinical and laboratory findings of these patients.

2. MATERIALS AND METHODS

1. Study population

This was a cross-sectional enrolled 60 newly SLE patients at the Center for Allergy - Clinical Immunology, Bach Mai Hospital from October 2022 to August 2023. SLE patients were diagnosed according to the criteria of Systemic Lupus International Collaborating Clinics (SLICC) with ≥ 4 criteria (at least 1 clinical criterion and 1 laboratory criteria) or LN proven on biopsy accompanied by the appearance of antinuclear antibodies (ANA) or anti-double-stranded DNA antibodies (anti-DsDNA). The patients who did not consent to participate or concomitant with other autoimmune diseases such as mixed connective tissue disease, scleroderma, rheumatoid arthritis, Sjogren's syndrome, primary APS, pregnant or taking any anticoagulants, were excluded from the study. The participant characteristics was shown in Table 1.

Table 1. General characteristics of the study subjects

Characteristics (n=60)	N	Mean/%
Age	60	41.82 ± 17.59 (years)
Female	52	86.7
Fever	41	68.3
Hair loss	14	23.3
Malar rash	22	36.7
Vasculitis	9	15
Oral ulcer	5	8.3
Arthritis	21	35
Serositis	34	56.7
Renal disorder	34	56.7
Neurologic disorder	10	18.3
Hemolytic disorder	51	85
Positive anti-ANA	57	95
Positive anti-dsDNA	60	100
SLEDAI – 2K	52	12.46 ± 5.98

2. Sampling data

Variables were collected according to the medical record form with biometric variables (age, gender, height, weight); symptoms and signs in SLE (butterfly rash, discoid rash, photosensitivity, oropharyngeal ulcers, hair loss, seizures, membrane effusion, SLEDAI index), kidney damage. Antibody tests were performed at the Center for Allergy and Clinical Immunology laboratory, Bach Mai Hospital, using ELISA Sandwich technique. In another hand, lupus anticoagulation test was performed on the ACLTOP 700 machine at the Center for Hematology and Blood Transfusion, Bach Mai Hospital.

3. Statistical analysis

Collect and process data using SPSS software version 20.0. Quantitative data were expressed as mean ± standard deviation. The threshold of statistical significance is determined when the index $p < 0.05$.

4. Research ethics

Research subjects were informed and voluntarily participated in our study. The information collected will be kept confidential and provided for research purposes. The study was approved by the Medical Ethics Committee of the Hanoi Medical University.

3. RESULTS

1. Characteristics of APS in research subjects

We firstly evaluated the clinical manifestations of APS in study subjects. We found that only 3 patients had history of thrombosis events (1 venous and 2 arterial) and 1 patient had ≥ 1 unexplained fetal death ≥ 10^{th} week of gestation. Regarding antibody profiling, LA positive was found in 30% patients; 61.7%, 35%, 18.33% patient positive for single, double and triple antibodies, respectively. The highest prevalence of positive antibody was 35.6% of aCL-IgM (Table 2 and Figure 1).

Table 2. Clinical manifestations and laboratory findings of APS

Clinical findings	**N**	**%**
Thrombosis	3	5
Venous thromboembolism	1	1.7
Arterial thromboembolism	2	3.3
Pregnancy morbidity	1	1.7
≥ 1 unexplained fetal death ≥ 10^{th} week of gestation	1	1.7
≥ 1 premature birth <34^{th} week of gestation because of:		
• *Eclampsia or severe pre-eclampsia*	0	0
• *Features of placental insufficiency*		
≥ 3 unexplained consecutive abortions < 10th week of gestation	0	0
Laboratory findings		
Single positive	37	61.7
Double positive	21	35
Triple positive	11	18.33
LA (+)	18	30

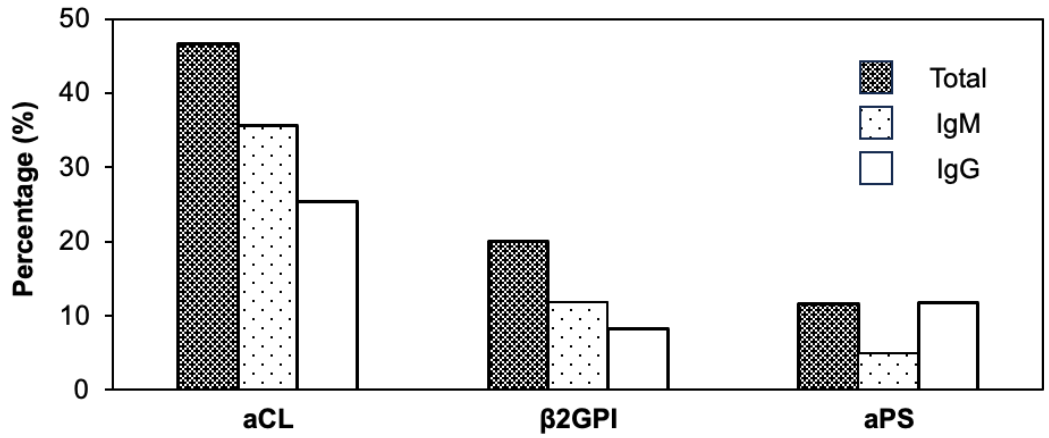

Figure 1. Antibody profiling of APS in study subjects

2. Relationship between antiphospholipid antibodies and some clinical and laboratory characteristics in SLE pateints

To evaluate the relationship between APS antibody profiling and other clinical and laboratory findings, the positive and negative group of each antibody were compared. Interestingly, patients with LA or any APS antibody positive were at high risk of having stage III to V as compared with negative group. We also found that the aPL (+) group showed a significantly higher number of malar rash as compared with aPL (-). Moreover, aCL-IgG (+) group had lower C3, but not C4 concentration than in negative group. There was no difference in pulmonary hypertension, thrombocytopenia, hemolytic anemia, arterial hypertension, 24-hour proteinuria between any positive and negative group (Table 3).

Table 3. Relation between vascular comorbidities, renal manifestations, and obstetric complications and aPL abs

Variable	Any aPL	LA	aCL -IgM	aCL -IgG	aβ2GPI-IgM	aβ2GPI-IgG	aPL -IgM	aPL -IgG
Pulmonary hypertension	ns	ns	ns	ns	ns	ns	Ns	Ns
Thrombocytopenia	ns	ns	ns	ns	ns	ns	Ns	Ns
Hemolytic anemia	ns	ns	ns	ns	ns	ns	Ns	Ns
Arterial hypertension	ns	ns	ns	ns	ns	ns	Ns	Ns
Chronic kidney disease, stage ≥ 3	0.03	0.03	ns	ns	ns	ns	Ns	Ns
24-hour proteinuria	ns	ns	ns	ns	ns	ns	Ns	Ns
Malar rash	0.012	ns	ns	ns	ns	ns	Ns	Ns
C3	ns	ns	ns	0.00	ns	ns	ns	Ns
C4	ns	ns	ns	ns	ns	ns	ns	Ns
SLEDAI	ns	ns	ns	ns	ns	ns	ns	Ns

4. DISCUSSION

In our study, we firstly estimated the prevalence of APS antibody profiling in newly SLE diagnosis in Vietnam. 61.7% SLE patients have positive for at least single APS antibody. Among that aCL was the most commonly found in our study subjects. Moreover, the relationship between these antibodies and the clinical, laboratory findings were also shown. Similar to epidemiological studies, SLE is commonly found in women and mainly in reproductive age, accounted for 86.7% of cases with an average age of 41.82 ± *17.59* years in our study. This may be explained by the important role of sex hormones, especially estrogen in the pathogenesis of the disease of SLE. Estrogen is reported to enhance CD8 levels, increase CD4 cytolytic activity, and the production of interferon-γ and interleukin-10 from helper T cells (Th)1 and Th2.[13] Kidney involvement is the most common clinical manifestation and mostly present with exacerbations with an average SLEDAI score of 12.46, which is explained by the fact that we only enrolled inpatients, but not outpatients.

In SLE, 30%-40% of patients are positive for aPL; when each aPL is investigated individually, the prevalence of a positive LA test and aCL varies between 11%-30% and 17%-40%, respectively.[14-17] In our study, the prevalence of patients positive for at least single antibody was 61.67%. The higher prevalence of positive APS antibodies might due to our study sampling design. Similarity, aCL positive were the most commonly found in APS antibody profiling comparable to other study in Vietnamese and worldwide population.[11,12,18] The relationship of APS antibody profiling with clinical manifestations and laboratory findings were also reported. Studies showed that aPL-positive has much higher prevalence of thrombocytopenia, autoimmune hemolytic anemia, arterial and/or venous thrombosis, pulmonary hypertension than aPL-negative SLE patients.[19-22] Unfortunately, we found no difference between these 2 groups in pulmonary hypertension, thrombocytopenia, hemolytic anemia, arterial hypertension, 24-hour proteinuria. This can be explained due to the sampling size and design. Interestingly, LA or any APS antibody positive were at high risk of having stage III to V as compared with negative group in our research. That might indicate that the presence of these antibodies would contribute to renal diseases in SLE patients. However, we could not explain whether the stage III to V due to lupus nephritis or APS nephropathy. APS nephropathy is a separate renal disease entity with different underlying pathogenic mechanisms compared to LN, the prevalence of APS nephropathy in SLE patients was reported as 32% and 23.2% in two studies.[23,24] According to a recently reported study encompassing a prospective cohort of 64 biopsy-proven active LN patients without concomitant APS nephropathy and cross-sectional analysis of 498 SLE patients with or without LN, both aPL positivity and level were similar in patients with active LN and non-renal SLE. However, IgG aCL or IgG aβ 2GPI positive patients had higher creatinine levels compared with patients without those IgG aPL both at active LN and after induction therapy, but not in the long-term data analyses. The authors concluded that aPL are not associated with occurrence of LN, and IgG aPL might contribute to an impaired renal function during a LN flare in the absence of APS nephropathy, probably due to their own pathogenic roles.[24] We suggest that aPL antibodies act as an independent factor associated with

increased the risk of chronic kidney disease.

CONCLUSION

Our study showed that aPL antibodies positive in 61.67% patients suffered from newly SLE diagnosis at the Center for Clinical Allergy and Immunology, Bach Mai Hospital. Among these, aCL antibody is the most common when identified in the blood of our research subjects. Positive aPL antibodies was independently associated with risk chronic kidney disease in SLE patients.

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