ORIGINAL ARTICLE

ROLE OF β-2 GLYCOPROTEIN 1, ANTI-CARDIOLIPIN AND LUPUS ANTICOAGULANT ANTIBODIES IN DIAGNOSIS OF ANTI-PHOSPHOLIPID SYNDROME

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Background: Anti-Phospholipid Syndrome (APS) is an autoimmune disease in which the patient is in a hypercoagulable state leading to vascular thrombosis and obstetric morbidity and mortality. The aim of my study is to see the presence of Anti-β2 Glycoprotein 1 (Anti-β2GP1), Anti-cardiolipin (aCL) and lupus anticoagulant antibodies (LA) in patients suspected of APS. **Methods:** This cross-sectional study was performed in the Department of Chemical Pathology, Liaquat National Hospital and Medical College, Karachi. A total of 115 patients from Rheumatology and Gynaecology Departments having thrombotic episodes were tested for the presence of one or more of the antibodies namely, Anti-β2GP1, Anti-Cardiolipin and Lupus Anticoagulant Antibodies in their sera. Data was analysed using SPSS-27. **Results:** Among 115 patients, 53.9% had no antibody positive and were considered to be disease free. Positivity with LA was seen in 23.5% of the population while 15.7% were positive with aCL IgM and 13% positive with aCL IgG. As regards Anti-β2GP1 IgM 8.7% and IgG 15.7% were positive in each assay. All parameters had low sensitivity but high specificity and negative predictive value. The diagnostic accuracy of aCL IgG and IgM was 77.3% and 74.7% respectively. **Conclusion:** Anti-β2GP1, aCL, and LA are valuable markers for the diagnosis of APS. They are better markers having a high specificity and negative predictive value.

Keywords: Anti-cardiolipin, Anti-phospholipid syndrome, Lupus anticoagulant antibodies, β2 glycoprotein 1

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INTRODUCTION

Antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized by a hypercoagulable state presenting with venous or arterial thrombosis and/or obstetrical complications.[1,2] These patients have circulating anti-phospholipid antibodies which cause thrombosis in arterial, venous, and even microvascular beds.[3,4] The deep veins of the legs and cerebral arteries are the two most common sites of thrombosis.[3] There are two types of APS: Type-1 is primary APS which is half of the cases, and Type-II is secondary APS in which the patient has other systemic auto-immune diseases.[5] Type-II APS can be seen in patients having Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA), Systemic Sclerosis, Sjögren's syndrome etc.[5,6] In 1999, international experts developed the Sapporo criteria for the diagnosis of APS which was later updated in 2006 in Sydney, Australia and was named the modified Sapporo or Sydney criteria.[7,8] In 2023 the ACR/EULAR APS classification criteria was developed which showed that at least one Anti-phospholipid antibody should be present in a patient with one APS clinical criterion and then added laboratory and clinical criteria were used to give weightage.[9] The clinical criteria include arterial, venous or small vessel thrombosis, or pregnancy complications which could be attributed to placental insufficiency.[7] The laboratory criteria involve the presence of at least one positive marker of APS at least 12 weeks apart, which include, positive Lupus anticoagulant (LA), Anti-Cardiolipin Antibody (aCL) IgG or IgM, or Anti-β2 Glycoprotein antibody (Anti-β2GP1) IgG or IgM.[7] If all three laboratory criteria are present with high titres of antibodies in a patient, that patient will have the maximal risk of developing APS.[10] Intermittent positivity of LA or low titres of the aCL and Anti-β2GP1 pose a low risk of development of the disease.[10] Our study aimed to see the role of different antibodies in diagnosis of APS and correlation of the antibodies.

MATERIAL AND METHODS

This analytical cross-sectional study was carried out in the Department of Chemical Pathology, Liaquat National Hospital and Medical College, Karachi from 2 Mar to 13 Sep 2024 after approval of Ethical Review Committee with Ref. No. RC-LNH-Pathology-01/2025/05. A total of 115 patients suspected of suffering from APS with either thrombosis or miscarriages were recruited in the study. The age, gender and other data was collected. Five mL blood was collected in gel top tube for Anti-β2GP1 and aCL antibodies. For LA, 3 mL blood was collected in blue

top tube containing sodium citrate as anticoagulant. Anti-β2GP1 was analyzed semi quantitatively by Quanta Lite ELISA from INOVA diagnostics, aCL was measured by EIA/ELISA method on ETI-MAX 3000 (EVOLIS) and LA w as measured by automated coagulation analyzer Sysmex CS-2500. For Anti-β2GP1 IgG and IgM, <15 U/mL was taken as negative, 15.0–39.9 U/mL as weakly positive, 40.0–79.9 U/mL as positive and ≥80 U/mL as strongly positive. For aCL IgM, <5.0 MPL-U/mL was taken as negative, 5.0–7.2 MPL-U/mL as equivocal, and >7.2 MPL-U/mL as positive. In case of aCL IgG, <10.0 GPL-U/mL was considered negative, 10.0–14.4 GPL-U/mL as equivocal, and >14.4 GPL-U/mL as positive. For LA, LA1 screening test, 31–44 seconds was taken normal, for LA2 confirmatory test, reference interval was 30–38 seconds. LA ratio ≤1.2 was taken as normal, 1.3–1.5 as weakly positive, 1.6–2.0 as moderately positive, and >2.0 was taken as strongly positive. Data analysis was done on IBM SPSS-27. Chi-square/Fisher exact test was applied, and $p \leq 0.05$ was considered significant.

RESULTS

Out of a total of 115 patients 79.1% were females 20.9% were males; 47% were aged ≤35 years, 30.4% were between 36 and 50 years, and 22.6% were >50 years old. Sixty-two (53.9%) patients had no antibody positive and were considered free of disease or had no risk of thrombosis; 31 (27%) had only one antibody positive, and 19 (16.5%) had two positive antibodies. There were just 3 (2.6%) cases having all three tests positive. Positivity with LA was seen in 23.5% of cases while 15.7% were positive with aCL IgM, and 13% positive with aCL IgG. Regarding Anti-β2GP1, IgM was positive in 8.7% and IgG in 15.7% (Table-1).

When LA positive and negative groups were compared, there were no significant differences in age, anti-β2GP1 IgG and IgM, aCL IgM levels, but significant differences were found in aCL isotype IgG titres (p=0.05) (Table-2). The sensitivity and specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy (DA) are tabulates as Table-3. All of these antibodies have a low sensitivity but high specificity and high NPV showing that they are better markers to rule out the disease than to diagnose the disease.

Table-1: Prevalence of different antibodies with isotypes

Antibodies Positive	IgG	IgM
Anti-β2GP1	18 (15.7%)	10 (8.7%)
aCL	15 (13.0%)	18 (15.7%)

Table–2: Comparison of lupus anticoagulant with age and bio-chemical markers (Mean±SD)

Parameters	Lupus Anticoagulant		*p*
	Positive	Negative	
Age (Years)	39.85±15.29	39.43±14.64	0.898
Anti-β2GP1 IgG (U/mL)	10.08±1.66	10.74±4.29	0.435
Anti-β2GP1 IgM (U/mL)	10.08±1.66	9.71±1.16	0.200
aCL IgM (MPL-U/mL)	9.86±13.32	4.75±5.74	0.063
aCL IgG (GPL-U/mL)	10.75±11.05	6.23±5.74	**0.050***

*Significant

Table-3: Diagnostic accuracy of Anti–β2 Glycoprotein (IgG, IgM) and Anti–Cardiolipin ((IgG, IgM)

	Lupus Anticoagulant		*p*	Sensitivity	Specificity	PPV	NPV	Diagnostic accuracy
	Positive	Negative						
β2Glycoprotein IgG								
Positive	4	14	1.000	14.8%	84.1%	22.2%	76.3%	67.82%
Negative	23	74						
β2Glycoprotein IgM								
Positive	4	6	0.241	14.8%	93.2%	40%	78.1%	74.78%
Negative	23	82						
Anti Cardiolipin IgG								
Positive	8	7	**0.007***	29.6%	92%	53.3%	81%	77.39%
Negative	19	81						
Anti Cardiolipin IgM								
Positive	8	10	**0.033***	29.6%	88.6%	44.4%	80.4%	74.78%
Negative	19	78						

Chi-square/Fisher exact test was applied, *Significant

DISCUSSION

The main antigen which is targeted by the antibodies in APS is β2GP1. This antigen is responsible for complement activation and homeostasis. If antibodies are formed against β2GP1, this can prevent homeostasis and complement activation and cause the thrombotic and obstetrical complications of APS.[11] Primary APS is characterized by the presence of thrombotic events or obstetrical complications and the presence of the antibodies to phospholipids.[12] Catastrophic APS has also been reported which showed the presence of the Anti-Phospholipid antibodies.[13] One South Asian study states that Catastrophic APS is more likely to occur in patients with triple antibody positivity.[14] This has also been validated by another study in which they say that the presence of all three antibodies, especially high titers of aCL and β2GP1 IgG are seen in Catastrophic APS.[15] According to Keeling *et al*, the most predictive test for thrombosis is LA, and if IgG β2GP1 or IgG aCL antibodies are also present in the patient, it will increase the specificity of the tests, and there is no role of IgM testing.[16] We found no significant differences between β2GP1 IgG or IgM in both LA groups. The presence of LA is the single most powerful predictor of thrombotic

risk and obstetric complications.[17] In our study 23.50% cases showed LA positive. If LA, β2GP1, and aCL are present in a patient, they are at highest risk of developing complications of pregnancy or thrombosis.[17] We found three cases in which all three antibodies were present, so these patients are at highest risk of developing these complications and/or Catastrophic APS. Díaz-Coronado *et al*[18] compared the thrombotic events and obstetrical complications between primary APS and secondary APS and found that thrombotic events were more common in the primary APS group. As regards obstetrical events like miscarriages, there were no significant differences between the two groups. Women with positive β2GP1 IgM were at three times increased risk of developing obstetrical complications like miscarriages. This is in contrast to the study by Keeling *et al*[16] who observed no role of IgM testing in APS. Mushtaq *et al*[14] found the IgM aCL to be the most common antibody found in the South Asian region, followed by IgG aCL and LA. We found LA to be the most common positive antibody, followed by aCL IgM and β2GP1 IgG respectively.

There were certain limitations to our study. These antibodies were not retested after 12 weeks as is the recommendation. This is probably due to the high financial burden in a low income country such as ours. The laboratory methods of measurement of these different antibodies were studied, and it was found that all the methods have their pitfalls.[19] LA is prone to interference by many substances, aCL and β2GP1 can be measured by a variety of methods, so causing a large variation of results.[19] This may also be true in our study.

CONCLUSION

Anti-β2GP1, Anti-cardiolipin and Lupus anticoagulant antibodies are valuable markers for the diagnosis of Anti-phospholipid syndrome. They are better markers to rule out the disease as they have a high specificity and negative predictive value.

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