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Prevalence of Anti-Cardiolipin and Anti-β2 Glycoprotein Antibodies in Indian Systemic Lupus Erythematosus Patients

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ABSTRACT

Anti-phospholipid antibodies (*APA*) *like anti-cardiolipin antibodies* (*ACA*) *and anti-β2glycoprotien* (*anti-β2 GP*) *are important cause of venous and arterial thrombosis and other occlusive vascular diseases. The prevalence of these antibodies in SLE patients at the time of diagnosis is not known in Indian SLE patients. This study was conducted to evaluate the prevalence of ACA and anti-β2 GP autoantibodies in SLE patients and to correlate them with disease activity and immune parameters such as C3, C4 and CRP levels, where 85 SLE patients referred from Rheumatology Department, KEM hospital, Mumbai were studied. SLE disease activity was evaluated by SLE Disease Activity Index* (*SLEDAI*) *score at the time of evaluation. All patients studied were in an active stage of disease of which 37.6% patients had renal disorders, which were categorized as Lupus Nephritis* (*LN*) *and 62.3% patients did not show any renal manifestations* (*non-LN*). *ACA and anti-β2 GP autoantibodies, to IgG and IgM subclasses were tested by ELISA. C3, C4 and CRP levels were detected by nephelometer. It was observed that 12.9% patients were IgG-ACA and IgM-ACA positive and ACA positivity was noted more among LN group Anti-β2 GP autoantibody positivity was 27.1% for IgG and 31.8% for IgM., IgG-anti-β2 GP antibodies were slightly higher in non-LN patients, whereas a higher incidence of IgM-anti-β2 GP antibodies were detected in LN patients. Hence detection both ACA and anti-β2 GP antibodies along with associated immune parameters were helpful to evaluate their possible association with disease severity in SLE patients. A long term follow up of patients having ACA and anti-β2 GP antibodies without thrombotic event is also needed to detect their possible thrombotic event in future along with their clinical presentation.*

***Keywords*: *Systemic Lupus Erythematosus* (*SLE*), *Anti-Cardiolipin Antibodies* (*ACA*), *Anti-β2 Glycoprotein Antibodies* (*anti-β2 GP*), *Lupus Nephritis* (*LN*), *SLE without Nephritis* (*Non-LN*)**

1. Introduction

Antiphospholipid antibody syndrome (APS) is perhaps one of the most confounding immunologic disorders. It's an acquired autoimmune disorder defined by the presence of antibodies against phospholipids. Anti-phospholipid antibodies (APA), namely the lupus anticoagulant (LAC) and the anti-cardiolipin antibodies (ACA) are a group of antibodies directed against negatively-charged phospholipid antigens (phosphatidylserine), on endothelial cell membranes and platelets. Previously these antibodies were thought to be recognizing epitopes on anionic phospholipids and a complex of lipid-bound human prothrombin [1]. Several components including high titres of APA, β2 GP-I and activation of endothelium or platelets, are now suspected to encompass the APS [2]. It seems likely that binding of APA on endothelial cells is mediated through the cofactor β2 GP-I [3]. The target antigen for could be a complex of β2 GP-I and anionic phosphoator lipids.

Systemic Lupus Erythematosus (SLE) is a chronic, multisystem, inflammatory disorder of autoimmune etiology, occurring predominantly in young women in their child-bearing age. Common manifestations may include arthralgias and arthritis, malar and other skin rashes, pleuritis or pericarditis, renal and CNS involvement and hematologic cytopenias. SLE may be precipitated by currently unknown environmental triggers that cause autoimmune reactions in genetically predisposed people [4,5]. Lupus Nephritis (LN) is histologically evident in

most patients with SLE with the involvement of varying degree of renal disease. Autoimmunity plays a major role in the pathogenesis of LN where autoantibodies form pathogenic immune complexes that deposit in kidneys. Glomerular thrombosis is another mechanism that may play a role in pathogenesis of LN, mainly in patients with APS and is believed to be the result of antibodies directed against negatively charged phospholipid-protein complexes [6,7]. APS is classified as primary or secondary depending on its association with other autoimmune disorders. Primary APS is diagnosed in patients demonstrating the clinical and laboratory criteria without other recognized autoimmune disease. Secondary APS is diagnosed in patients with other autoimmune disorders such as SLE. One-third of patients with SLE also have antiphospholipid antibodies, and approximately one-third of those with antibodies have clinical signs of antiphospholipid antibody syndrome [8].

Phospholipids such as cardiolipin, β2 glycoprotein and LAC are responsible for prevention of blood clotting. In patients with SLE who have bad obstetric history (BOH) or recurrent pregnancy loss (RPL), both cardiolipin and lupus anticoagulant antibodies are often present in high titre [9]. ACA may belong to both IgG and IgM subtypes. The IgG antibodies seem to be better predictors of fetal outcome. More recent studies suggest that the antibodies that really matter are those to β2 GP, the cofactor by which ACA binds to phospholipid and usually are present with ACA [10]. Earlier studies have confirmed that patients' positive for ACA are at risk of repeated episodes of thrombosis, fetal loss and thrombocytopenia. APA occurs in up to 60% of patients with SLE and may be of pathogenic significance in LN where the presence of intra glomerular capillary thrombosis has also been described [11-14].

Present study was designed to evaluate the prevalence of ACA and anti-β2 GP autoantibodies in SLE patients and to correlate them with disease activity and immune parameters.

2. Materials and Methods

This study was conducted in 85 SLE patients from Rheumatology department of KEM hospital, Mumbai, India over the period of 2 years (2008-2010). SLE patients were diagnosed according to the American College of Rheumatology (ACR) criteria [15]. This study was carried out after obtaining the requisite Ethics Committee approval and a written consent from the patient. The disease activity was assessed at the time of evaluation using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [16]. Pregnant and post menapausal women, smokers, patients with diabetes and patients with significant hyperlipemia were excluded. After blood collection, sera were stored in aliquots at –80˚C until tested. Renal biopsies of Lupus Nephritis (LN) cases were examined by light microscopy using hematoxylin, eosin, periodic Schiff (PAS) staining. Immunofluorescence microscopy was done using anti-IgG, anti-IgM, anti-IgA, anti-C3, anti-C4 and anti-fibrinogen fluorescein isothiocyanate conjugate (FITC). In LN patients the renal histology was classified according to WHO criteria [17]. Anti-Cardiolipin antibodies (ACA) to IgG and IgM isotypes and anti-β2 GP autoantibodies to IgG as well as IgM isotypes were detected by ELISA using commercially available kits (Euroimmune, Lubeck). C3, C4 and CRP levels were detected using a Nephelometer (BN ProSpec, Dade Behring, Germany). The Laboratory was blinded to the disease status of patients and their visceral involvement and a double blinded study was conducted on the autoantibody positive samples.

3. Results

A total 85 SLE patients were included in the study of which 80 were females and remaining 5 were males. The ages ranged between 16 yrs and 36 yrs with mean age being 26.8 ± 9.9 yrs. According to the ACR criteria, clinical manifestation of patients indicated that majority of these patients (60%) had arthritis followed by rash (Malar and Discoid) among 38.8% patients. It was observed that renal disorders were seen among 32/85 (37.6%) patients and these patients were categorized as LN. Remaining 53 patients (62.4%) did not showed renal manifestation and was categorized as non-LN. Other manifestations such as photosensitivity (20%), serositis (24.7%) and neurological disorder (16.5%) were noted among the study group. Laboratory findings indicated that all these patients were in active stage of disease where ANA positivity was noted among all. Hematological disorders showed anemia (Hb $\leq$ 7.0 g/dl) and/or autoimmune hemolytic anemia (AIHA) in 27.1%, leucopenia (WBC count $\leq$ 4.0 $\times$ 10^3/µl) in 16.5%, lymphopenia (total lymphocyte count $\leq$ 2.0 $\times$ 10^3/µl) in 11.8% and thrombocytopenia (platelet count $\leq$ 150 $\times$ 10^3/µl) in 25.9% patients. Immunological disorders showed raised CRP levels (>5 mg/L) in 51.8% patients where 61.4% patients had previous history of bacteria, viral or parasitic infections and remaining 38.6% patients had no history of previous infections. Decreased C3 levels alone (<90 - 180 mg/µl) were detected in 22.4% patients whereas in 13% patients, a decreased C4 levels alone (<10 - 40 mg/µl) were noted. Both C3 and C4 levels were reduced in 27.1% patients where as 37.7% patients had normal C3 as well as C4 levels (**Table 1**).

As shown in **Table 2**, it was observed that 12.9% patients had developed IgG-ACA and IgM-ACA individu ally each where as 3.5% of patients had developed both

Table 1. Baseline characteristics of SLE patients according to ACR criteria (n = 85).

Characteristics:	
Sex Ratio (F:M)	16:1
Mean Age (Years ± SD)	26.8 ± 9.9
Clinical Manifestations (%)	
Rash (Malar or Discoid)	33 (38.8%)
Photosensitivity	17 (20%)
Oral Ulcers	24 (28.2%)
Arthritis	51 (60%)
Serositis	21 (24.7%)
Renal Disorders	32 (37.6 %)
Neurological Disorders	14 (16.5%)
Laboratory Characteristics	
Hematological Disorders	
Anemia/ AIHA	23 (27.1%)
Leucopenia	14 (16.5%)
Lymphopenia	10 (11.8%)
Thrombocytopenia	22 (25.9%)
Immunological Disorders	
↑ CRP	44 (51.8%)
↓ C3 alone	19 (22.4%)
↓ C4 alone	11 (13%)
↓ C3 & C4 both	23 (27.1%)

Table 2. Anti-Cardiolipin (ACA) and anti-β2 glycoprotein (anti--β2 GP) antibodies in (n = 85).

SLE type	Anti-Cardiolipin antibodies (ACA) (n = 85)	Anti-β2 glycoprotien antibodies (anti-β2 GP) (n = 85)
Total SLE(n = 85)		
IgG positive	11 (12.9%)	23 (27.1%)
IgM positive	11 (12.9%)	27 (31.8%)
IgG + IgM positive	3 (3.5%)	9 (10.6%)
IgG negative	74 (87.1%)	62 (72.9%)
IgM negative	74 (87.1%)	58 (68.2%)
LN(n = 32)		
IgG positive	4 (12.5%)	8 (25%)
IgM positive	4 (12.5%)	**12 (37.5%)**
IgG + IgM positive	1 (3.1%)	3 (9.4%)
IgG negative	28 (87.5%)	24 (75%)
IgM negative	28 (87.5%)	20 (62.5%)
Non-LN(n = 53)		
IgG positive	7 (13.2%)	15 (28.3%)
IgM positive	7 (13.2%)	**15 (28.3%)**
IgG + IgM positive	2 (3.8%)	6 (11.3%)
IgG negative	46 (86.8%)	38 (71.7%)
IgM negative	46 (86.8%)	38 (71.7%)

IgG and IgM antibodies to ACA. When these patients were categorized further into LN and non-LN groups, ACA positivity for both IgG and IgM autoantibodies was slightly higher in non-LN group (13.2%) as compared to 12.5% in LN group. Anti-β2 GP positivity was 27.1% for IgG-β2 GP and 31.8% for IgM-β2 GP where as 10.6% of the patients developed anti-β2 GP antibodies to both IgG and IgM subclasses. Among LN and non-LN groups, IgG-β2 GP positivity revealed a slightly higher incidence (28.3%) in non-LN patients as compared to LN patients (25%) where as LN patients showed a higher incidence for IgM-β2 GP positivity (37.5%) as compared to 28.3% of patients in non-LN group. The cut off levels for IgG-ACA was 185 u/ml, IgM-ACA is 186 u/ml, IgG-β2 GP is 156 u/ml and IgM-β2 GP is 290 u/ml as per the normal individuals tested.

Table 3 gives the correlation of ACA and anti-β2 GP levels with SLEDAI and other immunological parameters such as CRP, C3 and C4. IgM-β2 GP positive LN patients had higher SLEDAI scores (mean ± SD; 20 ± 15.8) Out of 23 patients with IgG-anti-β2 GP positivity, 15 (65.2%) patients showed raised CRP levels where non-LN patients 11/15 (73.3%) had higher CRP levels as compared to 4/8 (50%) in LN group. Among IgM anti-β2 GP positive patients 17/27 patients (63%) showed reduced C3 and C4 levels, where in LN group 10/12 patients (83.3%) had reduced C3 and C4 levels as compared to 7/15 patients (46.7%) in non-LN group.

The distribution of clinical manifestations according to the ACR criteria among ACA and anti-β2 GP positive patients at the time of evaluation was as shown in **Table 4**. ACA positive patients showed a higher incidence for clinical manifestations such as malar and discoid rash, photosensitivity, oral ulcers, vasculitis, alopecia, fever, arthritis and myositis. A slightly higher incidence for clinical manifestations such as renal disorders, serositis, neurological and hematological manifestations such as leucopenia, thrombocytopenia and autoimmune hemolytic anemia (AIHA) were noted among anti-β2 GP patients. It was observed that among ACA positive patients, none of the patient had leucoepenia where as among anti-β2 GP positive patients having leucopenia, WBC counts ranged between (2.9 - 3.7) × 10^3/µl with mean ± SD value of 3.3 ± 0.6. In a group of ACA positive patients having thrombocytopenia, platelet counts ranged between (39 - 71) × 10^3/µL with a mean ± SD value 5.5 ± 22.6 where as anti-β2 GP patients having thrombocytopenia showed platelet counts ranged between (12 – 141) × 10^3/µl with a slightly higher mean ± SD value (58.1 ± 48.3). **Table 5** shows distribution of clinical severity categorized into mild, moderate and severe based on the SLEDAI scores.

4. Discussion

Anti-phospholipid antibodies (APA) are a distinct group of autoantibodies that appear in a variety of autoimmune diseases, particularly Systemic Lupus Erythematosus (SLE). They are associated with clinical events such as arterial and/or venous thrombosis, and obstetric complications with a strong association of ACA with thrombosis, thrombocytopenia, recurrent fetal losses and Coombs'

Table 3. Correlation of ACA and anti-β2 GP levels with SLEDAI and other immunological parameters.

SLE type	Anti-Cardiolipin antibodies (ACA)		Anti-β2 glycoprotien antibodies (anti-β2 GP)	
	IgG	IgM	IgG	IgM
Total SLE	11	11	23	27
Range	190 - 451	186 - 1232	156 - 410	297 - 3000
Mean + SD	265.1 ± 89.7	392.9 ± 299.7	198.1 ± 33.6	847 ± 683.2
SLEDAI				
Range	4 - 43	4 - 24	4 - 35	4 - 42
Mean + SD	14.6 ± 11.1	12.1 ± 5.9	14 ± 9.1	15.3 ± 10.1
↑ CRP	11	9	15	14
↓ C3 alone	3	0	3	0
↓ C4 alone	0	0	1	0
↓ C3 & C4 both	1	3	3	4
LN	4	4	8	12
Range	190 - 451	186 - 459	166 - 277	338 - 3000
Mean ± SD	261.3 ± 126.7	295.3 ± 117.0	212.3 ± 42.4	786.1 ± 756.8
SLEDAI				
Range	4-19	4 - 15	10 - 35	8 - 64
Mean ± SD	11.3 ± 7.3	10.8 ± 5.0	16.9 ± 8.6	20 ± 15.8
↑ CRP	4	2	4	6
↓ C3 alone	0	0	1	0
↓ C4 alone	0	0	1	0
↓ C3 & C4 both	0	1	1	2
Non-LN	7	7	15	15
Range	197 - 402	210 - 1232	156 - 410	297 - 2150
Mean ± SD	267.3 ± 73.4	448.7 ± 364.5	203.7 ± 61.5	895.3 ± 641.2
SLEDAI				
Range	4 - 43	4 - 24	4 - 43	4 - 42
Mean ± SD	16.6 ± 12.9	12.9 ± 6.6	12.9 ± 9.3	14.4 ± 10.2
↑ CRP	7	7	11	8
↓ C3 alone	3	0	2	0
↓ C4 alone	0	0	0	0
↓ C3 & C4 both	1	2	2	2

Table 4. Distribution of clinical manifestations according to ACR criteria among ACA and anti-β2 GP positive patients.

Clinical presentation	ACA positives (IgG and/or IgM) (n = 19)	Anti-β2 GP positives (IgG and/or IgM) (n = 41)
Malar Rash &/ Discoid Rash	**9 (43.4%)**	14 (34.1%)
Photosensitivity	**4 (21.1%)**	8 (19.5%)
Oral ulcers	**7 (36.8%)**	11 (26.8 %)
Arthritis	**14 (73.7%)**	24 (58.5%)
Serositis	6 (31.6%)	**14 (34.1%)**
Renal Disorders	3 (15.8 %)	**8 (19.5%)**
Neurological Disorders	2 (10.5%)	**8 (19.5%)**
Hematological Disorders	1 (5.3%)	**5 (12.1%)**
Systemic vascular thrombosis	4 (21.0%)	**10 (24.4%)**
Myositis	**7 (36.8%)**	10 (24.4%)
Alopecia	**8 (42.1%)**	16 (39.0%)
Fever	**4 (21.1%)**	7 (17.1%)

Table 5. Distribution of clinical severity based on the SLEDAI scores.

SLE type (SLEDAI)		Anti-Cardiolipin antibodies (ACA)				Anti-β2 glycoprotien antibodies (anti-β2 GP)			
		IgG		IgM		IgG		IgM	
		Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
		(4)	(18)	(3)	(19)	(6)	(16)	(7)	(15)
Mild (<8)	Mean ± SD	5 ± 1.1	6.6 ± 1.9	5.3 ± 2.3	6.4 ± 1.8	6.3 ± 2	6.2 ± 1.9	5.7 ± 1.8	6.5 ± 1.9
	Range	4 - 6	3 - 8	4 - 8	3 - 8	4 - 8	3 - 8	4- 8	3 - 8
		(4)	(37)	(7)	(34)	(13)	(28)	(12)	(29)
Moderate (8 - 18)	Mean ± SD	15 ± 2.6	12.1 ± 2.4	13.3 ± 2.9	12.5 ± 2.5	12.5 ± 2.1	12.3 ± 2.8	12.7 ± 2.6	12.2 ± 2.6
	Range	12 - 18	9 - 18	10 - 18	9 - 18	10 - 16	9 - 18	10 - 18	9 - 18
		(3)	(19)	(2)	(20)	(6)	(16)	(9)	(13)
Severe (>18)	Mean ± SD	27 ± 13.8	24.4 ± 7.2	22 ± 2.8	25 ± 8.3	26.2 ± 10.3	24.2 ± 7.3	26.2 ± 9.4	23.7 ± 7.1
	Range	19 - 43	20 - 42	20 - 24	19 - 43	19 - 43	18 - 42	18 - 42	19 - 43

positivity in SLE and related autoimmune disorders [18-22].

Recently Mostafa *et al.*, 2010 had reported an incidence of 16.7% for ACA among SLE patients [23]. Similar incidence was found in our study where IgG-ACA and IgM-ACA positivity was 12.9% each which was lower than anti-β2 GP autoantibody positivity (IgG-β2GP: 27.1% and IgM-β2 GP: 31.8%). Other studies such as Petri *et al.*, 2010 reported 47% ACA and 32.5% anti-β2 GP autoantibodies in SLE, Biggioggero *et al.*, 2010 had reported 16.5% IgG-ACA and 9.4% of IgM-ACA and an incidence of 4.7% IgG-β2 GP and 5.9% for IgM-β2 GP antibodies, Jallouli *et al.*, 2008 had reported 71.6% for ACA and Descloux *et al.*, 2008 had reported an incidence of 49% ACA [24,25,13,14]. In a study on South African SLE patients, Gould *et al.*, 2006 had reported a very high incidence of 53% and 84% for ACA and anti-β2 GP antibodies where as Al Arfaj *et al.*, 2009 had reported an incidence of 49.7% and 33.5% for IgG-ACA and IgM-ACA respectively among Saudi Arabian SLE patients [26,27]. Recently Woo *et al.*, 2010 had reported an incidence of 18.2% and 31.8% for IgG-ACA and IgM-ACA respectively and 5.7% for anti-β2 GP to IgG and IgM isotypes in Korean SLE patients. Shrivastav *et al.*, 2001 had reported 51% IgG-ACA and 44.7% IgG-β2 GP autoantibodies [28,29].

Thrombosis varies in SLE patients from 7.2 to 12%. Sarabi *et al.*, 2005 reported that the most frequent causes of death in active SLE are infection and thrombosis [30]. The risk of thrombosis for SLE patients reported to be significantly higher and due to the increased incidence of traditional cardiovascular and nontraditional lupus-related thrombosis risk factors, SLE patients are at significantly increased risk of premature atherosclerosis and/or thrombosis. The prevalence of vascular events in SLE patients ranges between 10% and 30%, for symptomatic coronary artery disease 6% - 20%, stroke 2% - 15%, and subclinical coronary artery disease 30% - 40% [31]. Our study showed a higher incidence of systemic vascular thrombosis in anti-β2 GP positive patients as compared to ACA positive patients with an equal distribution for venous and arterial thrombosis in both the groups.

Recurrent pregnancy loss (RPL) has been associated with APA including ACA and lupus anticoagulant. It had been reported that the risk of fetal loss is found to be increased in patients with hypertension, active SLE, LN, or abnormally low complement levels. Risk also increased for patients with APA: from 6% to 24% of patients with SLE are positive for LAC and 40% are positive for ACA [32-34]. In our study two patients had RPL and other two had BOH, but they did not show the presence of ACA or anti-β2 GP antibodies. Shrivastav *et al.*, 2001 reported that incidence of neurological disorders such as seizures were noted in 9.4% SLE patients which was significantly associated with the presence of ACA and anti-β2 GP antibodies [29]. Neurological disorders were seen more in anti-β2 GP positive patients than in ACA positive patients in our study.

Zheng H *et al.*, 2009 reported that LN patients had elevated SLEDAI as compared to non-LN having elevated renal tissue injury, high serum creatinine, BUN and proteinuria levels with lower serum C3 levels [9]. Bhandari *et al.*, 1998 had reported a significant reduction in C3 and C4 levels in ACA positive patients, with a strong relationship to disease activity/severity at presentation when compared with changes in ACA negative patients (p < 0.0%) where renal function at presentation was worse in patients with ACA positivity [35] It was suggested that ACA is a strong predictor for the presence of intra glomerular thrombi in patients with LN indicating the worse long term renal outcome in these patients. Our findings showed more complement consumption in

anti-β2 GP positive patients than ACA positives. Hence detection both ACA and anti-β2 GP antibodies along with associated immune parameters were found to be helpful parameters to evaluate their possible association with disease severity in SLE patients. A long term follow up of patients having ACA and anti-β2 GP antibodies without thrombotic event is required to detect their possible thrombotic event in future along with their clinical presentation.

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