Original Research Article

The Egyptian Journal of Immunology

The Egyptian Journal of Immunology
Volume 28 (4), 2021: 185–194.
www.Ejimmunology.org

Assessment of Anti-β2 glycoprotein1 antibody in Systemic Lupus Erythrematosus Patients

Taghreed M Kamal Eldin[1], Wafaa T Elsherif[1], Salwa S Elgendy[2], Mariam S youssif[1] and Tarek T. H. ElMelegy[1]

[1]Department of Clinical Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt.

[2]Department of Internal Medicine, Faculty of Medicine, Assiut University, Assiut, Egypt.

Corresponding author: Tarek T. H. ElMelegy, Laboratory of Clinical Immunology, Department of Clinical Pathology, Assiut University Hospital, Assiut University, Assiut, Egypt.
Email: tarek.elmelegy@aun.edu.eg.

Abstract

Secondary anti-phospolipid syndrome (APS) is diagnosed in many patients with systemic lupus erythrematosus (SLE) especially with thromboembolic events and/or pregnancy loss in the presence of persistent laboratory evidence for anti-phospholipid antibody (aPL). In this work, we aimed to detect the prevalence of IgG and IgA anti-β2 glycoprotein1 (β2GP1) in SLE patients. Serum samples were collected from 50 female patients with SLE (25 had APS and 25 patients who did not have APS), in addition to 22 apparently healthy females with matched age as a control group. All samples from patients and control were tested for lupus anticoagulant (LA), IgG and IgA isotypes of antiβ2GPI, Anti-nuclear antibody (ANA), Anti-double strand antibody (Anti-dsDNA). Number of patients positive for Anti-β2GP1 antibodies were significantly increased in APS patients compared to non-APS patients (*P*=0.015). Anti-β2GP1 IgA isotype was significantly higher in APS patients than in non-APS patients (*P*=0.011) and significantly correlated with deep venous thrombosis and pregnancy morbidity (*P*=0.004). There was no difference in anti-β2GPI IgG isotype between APS patients and non-APS patients. We concluded that although anti-β2GPI IgA is not within Sapparo diagnostic criteria, it seems to contribute to the pathogenesis of thrombotic manifestations of SLE and may represents a useful indicator particularly when standard aPL tests are negative.

Keywords: Systemic lupus eryhtrematosus, Anti-phosholipid syndrome, Anti-phospholipid antibodies, lupus anticoagulant

Date received: 15 July 2021; **accepted:** 22 August 2021

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that predominately affects women. It is characterized by a relapsing intermitting course with periods of flares, alternating periods of remission and broad spectrum of clinical manifestations, however, its course and organ involvement are unpredictable. Although over the last few decades an improvement in survival for SLE patients has been observed, pathogenic

mechanisms underlying this disease are still unclear. Co-morbidities due to the disease itself and treatment regimens, as well as multiple aspects of SLE, are under intensive investigation.[1]

SLE is characterized by involvement of large number of organs, presence of immune complexes in the affected organs and the excessive production of a variety of autoantibodies, including antiphospholipid antibodies (aPL).[2] Although SLE patients frequently harbor aPL, which significantly affects their clinical manifestations and prognosis, information about the characteristics of aPL positivity in SLE patients is insufficient. Previous studies of aPL in SLE may have not reflected the true prevalence of aPL, and their results were inconsistent. Many SLE patients produce aPL intermittently, and their levels vary depending on SLE disease activity, hindering accurate estimation of the prevalence of aPL. Also, the methods used to measure serum aPL levels and the cut off values for positivity could have varied among studies. In addition, a study results suggested that ethnic differences could influence the prevalence of aPL.[3]

One of the major complications of SLE is antiphospholipid syndrome (APS). It is a systemic auto-immune disease, characterized by venous and arterial thrombosis and often multiple recurrent fetal losses frequently accompanied by a moderate thrombocytopenia in the presence of a lupus anticoagulant, elevated anti-cardiolipin antibodies, and anti-beta-2-Glycoprotein-1 (anti-β2G1) antibodies. Diagnosis of the syndrome can be based on the presence of any of the clinical manifestations together with the detection of either of the aPL antibodies.[4]

It has been reported that the binding of aPL to cardiolipin requires the presence of β2GPI as a cofactor. β2GPI is an apo-lipoprotein, a member of the complement control family and is considered a natural inhibitor of coagulation.[5,6] In addition, *in vitro* studies have shown that it has anti-angiogenic and anti-apoptotic effects.[7,8] It consists of 326 amino acids, arranged in 5 highly homologous complement-control protein domains that are designated I to V from the N- to the C-terminus. Domain I (DI) is the epitope for the pathological autoantibodies.[5,9]

Currently routine diagnosis of APS includes testing for antibodies of the IgG or IgM isotype to β2GPI as a one of the three antibodies that are included in the criteria of diagnosis of APS.[10] These criteria do not include the IgA isotype for aPL and the role of IgA as a diagnostic marker for APS is still a matter of debate.

There are several suggested reasons for why the concomitant presence of SLE may increase the risk of thromboembolism in patients with anti-β2GP1 IgA.[11] Firstly, SLE may confer an increased risk for thrombosis that is independent from that of antiphospholipid antibodies[12] Secondly, concurrent inflammation and activation of innate immunity during flare of SLE may provide a second hit necessary for antiphospholipid antibody reaction to induce thrombosis.[13] Thirdly, the epitope specificity of anti-β2GPI IgA may differ depending on whether antiphospholipid antibodies arise in presence or absence of systemic autoimmunity.[14] The lack of well-designed prospective studies raises doubts as to the usefulness of testing for IgA aPL. Testing for IgA aPL may enhance thrombotic risk assessment only under certain circumstances, such as when clinical signs and symptoms of APS are present (usually in association with SLE), and tests for standard aPL are negative.[15] Consequently, this work aimed to clarify the significance of the different anti-ß2GPI isotypes (IgG and IgA) among Egyptian SLE patients with or without associated APS.

Subjects and Methods

The study included 50 SLE female patients, fulfilling criteria of the American college of rheumatology for diagnosis of SLE.[16] Patients were recruited, during the period from May 2015 to October 2016, at the Rheumatology Unit, Department of Internal Medicine, Assiut University Hospitals.

Exclusion criteria: Patients with risk factors of arterial and venous thrombosis as history of diabetes mellitus, patients with uncontrolled systemic hypertension, dyslipidemia were excluded.

All patients were subjected to careful medical history taking and clinical examination. SLE patients were classified into two groups; Group 1: 25 SLE patients with secondary APS diagnosed using the Sapporo classification criteria.[17] Group 2: 25 SLE patients without APS. Also, 22 apparently healthy females who were age matched to SLE patients were recruited as a control group.

Obstetric history was carefully taken from all study subjects with special emphasis on pregnancy morbidities (abortion, stillbirth and pre-eclampsia). The following laboratory investigations were done for all study subjects:

-Lupus Anticoagulant testing was performed using LA 1 screening reagent (Ref. no. OQGP17, Seimens Healthcare Diagnostics Products GmbH, USA) and LA2 Confirmation reagent (Ref. no. OQGR13, Seimens Healthcare Diagnostics Products GmbH, USA) on SysmexCA-1500 (Seimens Healthineers, Germany) according to manufacturer's instructions and LA ratio was calculated:

$$\text{LA Ratio} = \frac{\text{LA1 Screening reagent clotting time.}}{\text{LA2 Confirmation reagent clotting time.}}$$

Normal LA1/LA2 ratio: 0.8-1.2 [18]

-Antinuclear antibody (ANA), anti-double stranded DNA (anti-dsDNA), anti-ß2GPI IgA and anti-ß2GPI IgG were done in the Laboratory of Clinical Immunology - Assiut University Hospital, on Alegria (Orgentec Diagnostics GmbH, Germany). Alegria is an automated enzyme linked immunosorbent assay (ELISA)- based system, Alegria test strips use the sensotronic memorized calibration (SMC) technology. Testing was performed according to the manufacturer's instructions.

A. ANA was done using ANA detect test strips (ORG200, Orgentec, Germany) which are used for the qualitative screening of IgG class autoantibodies against SS-A -52 (Ro-52), SS-A-60 (Ro-60), SS-B (La), RNP/Sm, RNP-70, RNP-A, RNP-C, Sm-BB, Sm-D, Sm-E, Sm-F, Sm-G, Scl-70, Jo-1, dsDNA, ssDNA, polynucleosomes, mononucleosomes, histone complex, histones (H1, H2A, H2B, H3, H4), Pm-Scl-100 and centromere B in human serum or plasma samples.[19] Test results are represented as index; index value < 1.0 is negative, index value from 1.0 to 1.2 is borderline and index value of > 1.2 indicates positive results.

B. Anti-dsDNA was done using dsDNA screen test strips (ORG204S, Orgentec, Germany) which are used for the quantitative measurement of IgG, IgA and IgM class autoantibodies against dsDNA in human serum or plasma samples.[19] Measurement range is 0 – 200 u/ml. Levels ≥ 25 u/ml are interpreted as positive.

C. Anti-ß2GPI IgA was done using anti beta-2-Glycoprotein IgA test strips (ORG221A, Orgentec, Germany) which are used for the quantitative measurement of IgA class autoantibodies against ß2GPI in human serum or plasma samples.[20] Measurement range is 1-100 u/ml. Levels up to 5u/ml are interpreted as negative, levels 5-8 u/ml are borderline and levels above 8 u/ml indicates positive results.

D. Anti-ß2GPI IgG was done using anti beta-2-Glycoprotein IgG test strips (ORG221G, Orgentec, Germany) which are used for the quantitative measurement of IgG class autoantibodies against ß2GPI in human serum or plasma samples.[20] Measurement range and its interpretation are as described above for anti-ß2GPI IgA.

Statistical analysis

Statistical analysis was done using SPSS (Statistical Package for Social Science) version 19. Chi-square and Fisher Exact tests were used to compare between qualitative variables. Mann-Whitney test was used to compare between two quantitative variables (non-normally distributed). P-value of < 0.05 was considered statistically significant.

Results

Demographic data and pregnancy morbidity of SLE patients and control subjects are shown in Table 1. There was statistically significant increase in pregnancy morbidities (abortion, stillbirth, pre-eclampsia) in SLE patients (60%) compared to that in control subjects (29.6%), *P*=0.035.

Table 1. Demographic data and pregnancy morbidity of SLE patients and control subjects.

	SLE Patients (n= 50)	Control (n= 22)	*P*-value
Age:(years)			
Mean ± SD	32.32 ± 7.71	31.55 ± 5.12	NS
Median (Range)	30.0 (18.0 - 49.0)	27.0 (20.0 - 46.0)	
Parity:			
Mean ± SD	2.81 ± 1.24	2.15 ± 0.93	NS
Median (Range)	3.0 (0.0 - 7.0)	2.0 (0.0 – 5.0)	
History of pregnancy	40 (80.0%)	17 (77.3%)	NS
Pregnancy morbidity:	24/40 (60.0%)	5/17 (29.4%)	0.035

SLE= Systemic lupus erythematosus, n= number, SD= standard deviation. *P*>0.05 is not significant (NS).

When comparing SLE patients to control subjects regarding the immunological laboratory features, none of the control subjects had a positive result in any of the tests (ANA, anti-dsDNA, LA ratio, anti-ß2GPI IgA and anti-ß2GPI IgG). The frequency of positive test results in SLE patients was 18/50 (36%) for LA. Thirty-four patients (68%) had positive anti-ß2GPI test result; 7 patients (14%) had positive anti-β2GPI IgG, 13 patients (26%) had positive anti-β2GPI IgA, 14 patients (28%) had positive results of both anti-β2GPI IgG and IgA (Figure 1). Levels of anti-dsDNA, LA ratio, anti-ß2GPI IgA and anti-ß2GPI IgG were significantly higher in SLE patients than in control subjects (Table 2).

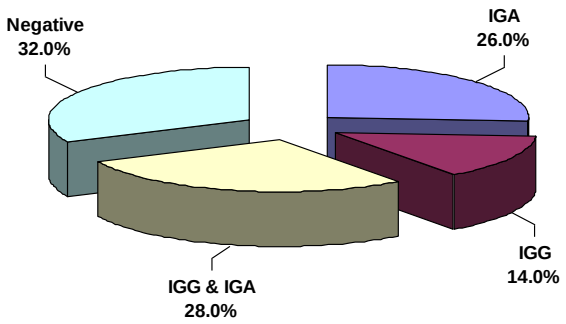

Figure 1. Distribution of anti-β2GP1 antibodies among systemic lupus erythromatosus patients.

Table 2. Levels of different antibodies in SLE patients and control subjects.

	SLE Patients (n= 50)	Control (n= 22)	*P*-value
Anti- dsDNA (u/ml)			
Mean ± SD	145.02 ± 161.60	9.47 ± 2.09	0.000
Median (Range)	72.1 (2.4 - 853.5)	9.4 (4.5 - 14.2)	
LA (ratio)			
Mean ± SD	1.28 ± 0.52	0.76 ± 0.13	0.000
Median (Range)	1.2 (0.3 - 2.9)	0.8 (0.5 - 1.0)	
anti-β2GPI IgG (u/ml)			
Mean ± SD	9.28 ± 8.30	1.58 ± 0.38	0.000
Median (Range)	7.7 (1.2 - 53.4)	1.5 (0.9 - 2.6)	
anti-β2GPI IgA (u/ml)			
Mean ± SD	20.20 ± 35.07	1.78 ± 0.35	0.000
Median (Range)	9.7 (0.8 - 160.0)	1.9 (1.0 - 2.5)	

SLE= Systemic lupus erythematosus, n= number, SD= standard deviation, anti-dsDNA= anti-double stranded DNA, LA= Lupus Anticoagulant, anti-β2G1= anti-beta-2-Glycoprotein-1. .*P*<0.05 is significant.

Table 3 shows demographic data and thrombotic complications in the two SLE patients' groups. Disease duration was significantly longer in the SLE patients with secondary APS (group 1) with mean± SD 5.06 ±3.32 years than in the SLE patients without APS (group 2) with mean± SD 2.97± 2.57 years. Pregnancy morbidity (which include abortion, stillbirth, preeclampsia) was significantly higher in group 1 (86.4%) than in group 2 (27.8%), *P*<0.0001. When comparing thrombotic complications between the two groups, there was a significant increase in the frequency of venous thrombosis in group 1 (80%) than in group 2 (12%); there was significant increase in deep venous thrombosis (DVT) in group 1 (60%) than group 2 (8%) and pulmonary embolism was significantly increased in group 1 (32%) than in group 2 (4%).

Table 3. Demographic data and thrombotic complications in SLE patients' groups.

	Group 1 (APS) (n= 25)	Group 2 (Non APS) (n= 25)	P-value
Age (years)			
Mean ± SD	33.44 ± 6.90	31.20 ± 8.44	NS
Median (Range)	34.0 (21.0 - 48.0)	29.0 (18.0 - 49.0)	
Duration of disease (years)			
Mean ± SD	5.06 ± 3.32	2.97 ± 2.57	0.009
Median (Range)	4.0 (0.5 - 14.0)	2.0 (0.1 - 10.0)	
Parity			
Mean ± SD	2.91 ± 1.20	2.70 ± 1.30	NS
Median (Range)	3.0 (0.0 - 5.0)	3.0 (0.0 - 7.0)	
History of pregnancy	22 (88.0%)	18 (72.0%)	NS
Pregnancy morbidity	19/22 (86.4%)	5/18 (27.8%)	0.000
Venous thrombosis:	20(80%)	3(12%)	0.000
DVT	15 (60 %)	2 (8 %)	0.000
Pulmonary embolism	8 (32 %)	1 (4 %)	0.023

APS= anti-phospolipid syndrome, n= number, SD= standard deviation, DVT= deep venous thrombosis.
P>0.05 is not significant (NS).

Frequency of positive anti-β2GPI IgA testing was significantly higher in the SLE patients with secondary APS (group 1) (72%) than in SLE patients without APS (group 2) (36%). However, there was no difference observed in the frequency of anti-β2GPI IgG positive tests in group 1 when compared to group 2 (Table 4). Anti-β2GPI IgA level was significantly higher in group 1 than in group 2, *P*=0,005 (Table 5).

Table 4. Frequency of positive tests in the two SLE patients' groups:

	Group1 (APS) (n= 25)		Group 2 (Non-APS) (n= 25)		*P*-value
	No.	%	No.	%	
LA ratio					
Positive (> 1.2)	12	48.0	6	24.0	NS
Negative	13	52.0	19	76.0	
Anti-β2G1 IgG					
Positive (> 8u/ml)	13	52.0	8	32.0	NS
Negative	12	48.0	17	68.0	
Anti-β2G1 IgA					
Positive (> 8u/ml)	18	72.0	9	36.0	0.011
Negative	7	28.0	16	64.0	
ANA					
Positive (index > 1.2)	20	80.0	20	80.0	NS
Negative	5	20.0	5	20.0	
Anti-dsDNA					
Positive (≥ 25 u/ml)	22	88.0	19	76.0	NS
Negative	3	12.0	6	24.0	

APS= anti-phospolipid syndrome, n= number, SD= standard deviation, LA= lupus anticoagulant, anti-β2G1= anti-beta-2-Glycoprotein-1, ANA= antinuclear antibodies, anti-dsDNA= anti-double stranded DNA. .*P*>0.05 is not significant (NS).

Table 5. Level of different antibodies in the two SLE patients' groups.

	Group1 (APS) (n= 25)	Group2 (Non-APS) (n= 25)	P-value
LA ratio			
Mean ± SD	1.38 ± 0.63	1.18 ± 0.35	NS
Median (Range)	1.2 (0.4 - 2.9)	1.1 (0.3 - 2.2)	
Anti-β2G1 IgG (u/ml)			
Mean ± SD	9.94 ± 9.89	8.61 ± 6.49	NS
Median (Range)	8.2 (1.7 - 53.4)	7.6 (1.2 - 28.9)	
Anti-β2G1 IgA (u/ml)			
Mean ± SD	25.56 ± 36.12	14.84 ± 33.86	0.005
Median (Range)	14.5 (1.4 - 160.0)	3.6 (0.8 - 145.0)	
Anti ds-DNA (u/ml)			
Mean ± SD	121.05 ± 182.91	168.99 ± 136.61	NS
Median (Range)	45.4 (7.4 - 853.5)	146.1 (2.4 - 409.0)	

APS= anti-phospolipid syndrome, n= number, SD= standard deviation, LA= lupus anticoagulant, anti-β2G1= anti-beta-2-Glycoprotein-1, anti-dsDNA= anti-double stranded DNA. .*P*>0.05 is not significant (NS).

Pregnancy morbidity and DVT frequencies were significantly increased in anti-β2GPI IgA positive SLE patients (66.7% and 51.9%, respectively) when compared to those with negative anti-β2GPI IgA test (26.1% and 13%, respectively), *P*=0.004. Frequency of pulmonary embolism

was significantly increased in anti-β2GPI IgG positive SLE patients (38.1%) when compared to those with negative anti-β2GPI IgG test (3.4%), *P*=0.002. Also, pregnancy morbidity frequency was significantly increased in SLE patients with positive LA test (66.7%) compared to those with negative LA test (37.5%), *P*=0.048 (Table 6).

Table 6. Relation between positive anti-phosphoilpid antibodies tests and clinical complications.

	Pregnancy morbidity (n=24)	Pulmonary embolism (n=9)	DVT (n=17)
LA			
Positive (n=18)	12 (66.7%)	5 (27.8%)	9 (50.0%)
Negative(n=32)	12 (37.5%)	4 (12.5%)	8 (25.0%)
P- value	0.048	NS	NS
Anti-β2G1 IgG			
Positive (n=21)	11 (52.4%)	8 (38.1%)	7 (33.3%)
Negative(n=29)	13 (44.8%)	1 (3.4%)	10 (34.5%)
P- value	NS	0.002	NS
Anti-β2G1 IgA			
Positive (n=27)	18 (66.7%)	4 (14.8%)	14 (51.9%)
Negative(n=23)	6 (26.1%)	5 (21.7%)	3 (13.0%)
P-value	0.004	NS	0.004

DVT= deep venous thrombosis, n= number, LA= lupus anticoagulant, anti-β2G1= anti-beta-2-Glycoprotein-1.
P>0.05 is not significant (NS).

Discussion

In this study most of SLE appears during the childbearing years in women, mainly between ages 15 and 50 years, thus reflecting a hormonal influence in disease pathogenesis.[21]

As regard pregnancy morbidity, it was significantly higher in SLE patients when compared to control subjects. This may be related to clinical or subclinical inflammation, presence of auto-antibodies, hormonal dysfunction, immune alteration in SLE and impairment of early placental development which lead to poor vascularization resulting in placental ischaemia and subsequent endothelial damage. According to the extent of this damage, pregnancy loss, intra uterine growth retardation, and/or preeclampsia can develop.[22,23] The pregnancy morbidity was higher in group 1 (SLE with APS) than in group 2 (SLE without APS). This is similar to what was reported by Tebo and colleagues.[24]

Also, in this study, longer disease duration and higher frequency of venous thrombosis (DVT and pulmonary embolism) was found in group 1 than in group 2. This observation agrees with the findings of Hamadani and colleagues, 2015,[25] who reported that DVT was present in 3% of SLE patients compared to 27.6% of SLE/APS patients, pulmonary embolism was 3% in SLE patients compared to 34.5% in SLE/APS patients, and abortion was 7.5% of SLE patients compared to 27.6% of SLE/APS patients and they correlated the increased duration of the disease to higher frequency of complications.

None of the control subjects had anti-β2GPI IgG or anti-β2GPI IgA. This result coincides with the result reported by Engel and his colleagues, 2017.[26]

In this study, anti-β2GPI antibodies were detected in 68%, and LA was detected in 36% of SLE patients. This in agreement with Engel and colleagues, 2017.[26] who reported that total anti-β2GPI antibodies was detected in 63.6% and LA

was detected in 45%. In another study, LA was detected in 25%.[25]

Anti-β2GPI IgA was more frequent than anti-β2GPI IgG (54% versus 42%) in SLE patients (14% of SLE patients had only IgG isotype, 26% had only IgA isotype, 28% had both IgG and IgA isotypes). In agreement with this finding, Rodriguez-Garcia and colleagues, 2015,[27] performed a systematic review of 16 studies and reported 56.3% prevalence of anti-β2GPI IgA antibodies in APS patients. However, Pericleous and colleagues, 2016,[28] reported that both anti-β2GPI (IgG and IgA) were detected in 8% of SLE patients.

Anti-β2GPI (IgG and IgA) was detected in 84% in patients with APS, in 52% of patients without APS, and LA was detected in 48% of APS group and 24% of non-APS group. In agreement with our results, Zhang and his coworkers, 2016, revealed that anti-β2GPI was found in 74% of cases in SLE/APS and in 19% of cases in SLE/non-APS.[29]

In this work, we detected anti-β2GPI IgG in 52 % in APS group and 32% in non-APS group and anti-β2GPI IgA in 72% in APS group and 36% in non-APS group. IgA was significantly higher in APS group than in non-APS group. Pericleous and colleagues, 2016,[28] reported that prevalence of anti-β2GPI IgG in APS was 65%, and prevalence of IgA anti-β2GPI in APS was 46%. The higher frequency of anti-β2GPI antibodies in the APS group relative to the non-APS group may be related to that antibodies against the domain I of β2 glycoprotein I (β2GPI) are increasingly recognized as the major pathogenic mechanism in APS.[30, 31] β2GPI is localized on the cell surface of human endothelial cell (EC) associated with lipids or membrane proteins, so, anti-β2GPI antibodies can activate EC, upregulate adhesion molecules, and induce cytokine production.[30]

The level of anti-β2GPI IgA was significantly higher in APS when compared to non-APS. In agreement with our results, Mcdonnnell and colleagues, 2020, stated that IgA positivity occurred more frequently and at higher titers in SLE patients with APS manifestations.[10]

In this work, comparison between APL isotype positive patients and negative patients regarding the obstetric morbidity, revealed that pregnancy complications were significantly associated with LA 66.7% (P=0.048) and anti-ß2GPI IgA 66.7% (P=0.004) and not with anti-ß2GPI IgG. In agreement with our results, Zhang and colleagues, 2016,[29] also found no significant association between anti-ß2GPI IgG and pregnancy morbidity.

Pulmonary embolism was significantly increased in anti-β2GPI IgG positive SLE patients (P=0.002), and DVT was significantly increased in anti-β2GPI IgA positive SLE patients (P=0.004). aPL is thought to interfere with the function of binding proteins and activate endothelial cells inducing a pro-inflammatory and pro-coagulant state in blood vessels which leads to thrombosis.[30, 31] The occurrence of thrombosis in patients with APS has been explained by several pathophysiological mechanisms, including activation of various cells (endothelial cells, monocytes and platelets),[32] acquired activated protein C resistance, [33] tissue factor expression and reduced tissue factor pathway inhibitor (TFPI),[34] complement activation[35] and resistance to the action of annexin A5.[36]

In this study, anti-β2GPI IgA isotype had the strongest association with APS and SLE manifestations and APS complications. These results confirm clinical significance for IgA aPL in predicting thrombotic events previously reported by Mcdonnnell and colleagues, 2020.[10]

In conclusion, anti-β2GPI antibodies were highly represented in SLE patients in general and in APS patients specifically. Anti-β2GPI IgA was the most antibody isotype, showing positivity in the study lupus patients. Anti-2GPI IgA level and number of patients positive for anti-β2GPI (IgA) were significantly higher in APS patients than in non-APS patients. Although anti-β2GPI IgA is not within Sapparo diagnostic criteria, it seems to contribute to the pathogenesis of thrombotic and non thrombotic manifestations of SLE and APS including DVT and pregnancy morbidity. Testing for anti-β2GPI IgA may enhance thrombotic risk assessment in association with SLE and may represent a useful indicator particularly when standard aPL tests are negative.

Author Contributions

WTE, TMK and SSE proposed and designed the study. SSE clinically evaluated the patients and followed them up. MSY collected patients' information and performed the laboratory work. TMK, TTHE and MSY interpreted the laboratory test results and analyzed the data. TMK and MSY wrote the paper draft. WTE, SSE and TTHE revised the paper. TTHE prepared the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

This research did not receive any specific grant either from Faculty Grant Office or other funding agencies.

Ethical approval

The study protocol was reviewed and approved by the Ethical Committees at Faculty of Medicine, Assiut University (IRB: 17101317, February 2015).

Informed consent

A signed consent form was obtained from each study participant.

References

1. La Paglia, G.M.C., Leone, M.C., Lepri, G., et al. (2017) One year in review 2017: systemic lupus erythematosus. *Clinical and Experimental Rheumatology*, 35(4):551-561
2. Garabet, L., Gelboil, L., Mowenkil, M.C., et al. (2016) Antiphospholipid antibodies are associated with low levels of complements C3, C4 in patients with systemic lupus erythromatosus. *Scandinavian Journal of Immunology*, 84: 95-99.
3. Seoung, W.N., Soo-Kyung, C., Dam, K., et al. (2018) Antiphospholipid Antibody Positivity and the Clinical Outcomes of Patients with Systemic Lupus Erythematosus. *Journal of Rheumatic Diseases*, 25 (4): 239-247
4. Asherson, R.A., Cervera, R., Piette, J., et al. (2018) The Antiphospholipid Syndrome: History, Definition, Classification, and Differential Diagnosis. In: Asherson, R.A., Cervera, R., Piette, J., Shoenfeld, Y., eds. The Antiphospholipid Syndrome. First edition. CRC Press (Reissued), 3-12.
5. Arachchillage, D.R.J., Laffan, M. (2017) Pathogenesis and management of antiphospholipid syndrome. *British Journal of Haematology*, 178 (2):181-195.
6. Mcneil, H.P., Simpson, R.J., Chesterman,C.N., et al. (1990) Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: beta 2-glycoprotein I (apolipoprotein H). Proc. Natl. Acad. Sci. U.S.A, 87, 4120-4
7. Yu, P., Passam, F.H., Yu, D.M., et al. (2008) β2-glycoprotein I inhibits vascular endothelial growth factor and basic fibroblast growth factor induced angiogenesis through its amino terminal domain. J Thromb Haemost; 6:1215–23.
8. Maiti, S.N., Balasubramanian, K., Ramoth, J.A., et al. (2008) β-2-glycoprotein 1-dependent macrophage uptake of apoptotic cells: binding to lipoprotein receptor-related protein receptor family members. J Biol Chem, 283:3761–6.
9. Agar, C., van Os, G.M., Morgelin, M., et al. (2010). Beta2-glycoprotein I can exist in 2 conformations: implications for our understanding of the antiphospholipid syndrome. Blood, 116,1336.-43.
10. McDonnell, T., Wincupa, C., Buchholzb, I., et al. (2020) The role of beta-2-glycoprotein I in health and disease associating structure with function: More than just APS. *Blood Reviews.* https://doi.org/10.1016/j.blre.2019.100610
11. Sweiss, N.J., Bo, R., Kapadia, R., et al. (2010) IgA anti-β2-glycoprotein I autoantibodies are associated with an increased risk of thromboembolic events in patients with systemic lupus erythematosus. *PLoS One*, 5(8): 122-180.
12. Romero-Diaz, J., Garcia-Sosa, I., Sanchez-Guerrero, J. (2009) Thrombosis in systemic lupus erythematosus and other autoimmune diseases of recent onset. J Rheumatol 36: 68–75.
13. Levine, J.S., Branch, D.W., Rauch, J. (2002) Anti-phospholipid syndrome. N Engl J Med 346: 752–763.
14. de Laat, B., Derksen, R.H., Urbanus, R.T., et al. (2005) IgG antibodies that recognize epitope Gly40-Arg43 in domain I of beta 2-glycoprotein I cause LAC, and their presence correlates strongly with thrombosis. Blood 105: 1540–1545.
15. Sciascia, S., Amigo, M., Roccatello, D., et al. (2017) Diagnosing antiphospholipid syndrome: 'extra-criteria' manifestations and technical advances. *Nat Rev Rheumatol*, 13: 548–560.
16. Hochberg, M.C. (1997) Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum*, 40:17-25.

17. Miyakis, S., Lockshin, M.D., Atsumi, T., et al. (2006) International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J ThrombHaemost*, 4:295–306.
18. Exner, I., Papadopoulos, G., Koutts, J. (1990) Use of a simplified Dilute Russell`s viper venom time (DRVVT) confirm heterogenicity among lupus anticoagulants. *Blood coag fibrinol*, 1(3):259-266.
19. Alba, P., Bento, L., Cuadrado, M.J., et al. (2003) Anti-dsDNA, anti-Sm antibodies, and the lupus anticoagulant: significant factors associated with lupus nephritis. *Ann Rheum Dis*, 62(6):556-560.
20. Banzato, A., Pozzi, N., Frasson, R., et al. (2011) Antibodies to Domain I of beta(2)Glycoprotein I are in close relation to patients risk categories in Antiphospholipid Syndrome (APS). *Thromb Res*, 128 (6):583-600.
21. Oktem, O., Guzel, Y., Aksoy, S., et al. (2015) Ovarian Function and Reproductive Outcomes of Female Patients With Systemic Lupus Erythematosus and the Strategies to Preserve Their Fertility. *Obstetrical And Gynaecological Survey*, 70 (3):196-210
22. Ostensen, L., Monika, G., Clowse, L., et al. (2013) Pathogenesis of pregnancy complications in SLE. *Current Opinion in Rheumatology*, 25 (5): 591-596.
23. Ünlü, O., Zuily, S., Erkan, D. (2016) The clinical significance of antiphospholipid antibodies in systemic lupus erythematosus. *Eur J Rheumatol*, 3: 75-84.
24. Tebo, A.E., Willis, R., Jaskowski, T.D., et al. (2016) Clinical significance and correlations between anti-β2 glycoprotein I IgA assays in antiphospholipid syndrome and/or systemic lupus erythematosus. *Clin Chim Acta*, 460:107-113.
25. Hamdani, M.A., Al-Arfaj, A.R.S., Parvez, K., et al. (2015) Pulmonary manifestations of systemic lupus erythematosus patients with and without antiphospholipid syndrome. *Pak J Med Sci*, 31(1):70-75.
26. Engel, B., Gregor, M., Beate, C., et al. (2017) Serum of patients with antiphospholipid syndrome induces adhesion molecules in endothelial cells. *Atherosclerosis*, supplement 30: 141-148
27. Rodriguez-Garcia, V., Ioannou, Y., FernandezNebro, A., et al. (2015) Examining the prevalence of non-criteria antiphospholipid antibodies in patients with antiphospholipid syndrome: a systematic review. *Rheumatology* (Oxford), 54: 2042–2050.
28. Pericleous, C., Ferreira, I., Borghi, O., et al. (2016) Measuring IgA Anti-β2-Glycoprotein I and IgG/IgA Anti-Domain I Antibodies Adds Value to Current Serological Assays for the Antiphospholipid Syndrome. *PLoS ONE*, 11(6):e0156407
29. Zhang, S., Wu, Z., Chen, S., et al. (2016) Evaluation of the diagnostic potential of antibodies to beta2- glycoprotein 1 domain 1 in Chinese patients with antiphospholipid syndrome. *Scientific Reports*, 6: 238-239.
30. Brandt, K.J., Kruithof, E.K., de Moerloose, D. (2013) Receptor involved in cell activation by antiphospholipid antibodies. *Thrombosis Research,* 132 (4): 408–413.
31. Belizna, C., Stojanovich, L., Cohen-Tervaert, J.W., et al. (2018) Primary antiphospholipid syndrome and antiphospholipid syndrome associated to systemiclupus: Are they different entities? *Autoimmunity Reviews*, 17(8): 739-745.
32. Urbanus, R.T., Derksen, R.H., de Groot, P.G. (2008) Platelets and the antiphospholipid syndrome. *Lupus,* 17:888–94.
33. Liestol, L., Sandset, P.M., Mowinckel, M.C., et al. (2007) Activated protein C resistance determined with a thrombin generation-base test is associated with thrombotic events in patients with lupus anticoagulants. *J Thromb Haemost*, 5:2204–10.
34. Kinev, A.V., Roubey, R.A. (2008) Tissue factor in the antiphospholipid syndrome. Lupus, 17:952–8.
35. Pierangeli, S.S., Girardi, G., Vega-Ostertag, M., et al. (2005) Requirement of activation of complement C3 and C5 for antiphospholipid antibodymediated thrombophilia. *Arthritis Rheum, 5*2:2120–4.
36. De Laat, B., Wu, X.X., van Lummel, M., et al. (2007) Correlation between antiphospholipid antibodies that recognize domain I of beta2-glycoprotein I and a reduction in the anticoagulant activity of annexinA5. *Blood*, 109:1490–4.