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## Prevalence of anti-Cardiolipin and anti- $\beta_2$ glycoprotein-1 antibodies in Systemic Lupus Erythematous Patients.

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### ABSTRACT

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease associated with a variety of antiphospholipids (aPL) such as anti-cardiolipin (aCL) and anti -  $\beta_2$  glycoprotein-1 ( $\alpha\beta_2$ Glyp1) antibodies. . A total of 115 patients (112 males and 3 females) were referred to Rheumatology Clinic at National Ribat University hospital, Medical Military Hospital, and Private Clinic in Khartoum state, Plasma aCL and  $\beta_2$  glycoprotein I antibodies were measured using ELISA technique. The prevalence of aCL autoantibody among patients was 44 (38.2 %), higher prevalence in females 42 (95 %) in contrast to males 5 % ( 2/44). Low, medium and high titer of aCL were 23 (52 %), 18(41 %), and 3(7. 0 %) respectively. The prevalence of  $\alpha\beta_2$ Glyp1 autoantibody among patients was 25(21.7 %), higher prevalence in females 24(96 %) compared to males 1(4 %).  $\alpha\beta_2$ Glyp1 low titer 4(16 %), medium titer 14(56%), and high titer 7(28 %) were determined. aCL were found more frequently 44 (38.2 %) than anti-  $\alpha\beta_2$ Glyp1antibodies25 (21.7 %), both markers are elevated in females 42 (95 %), 24(96 %) than males 5 % ( 2/44), 1(4 %) respectively. aCL and  $\alpha\beta_2$ Glyp1antibodies are very helpful markers in diagnosis of SLE and useful to minimize the risk for thrombosis.

**Keywords:** aCL, anti  $\beta_2$  Glycoprotein-1, SLE, antiphospholipids.

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## INTRODUCTION

Antiphospholipid antibodies (aPL) are a heterogeneous group of autoantibodies that include anticardiolipin (aCL), lupus anticoagulant (LA) and  $\text{a}\beta_2\text{Glyp1}$  are a group of antibodies directed against negatively-charged phospholipid antigens [1]. The pathogenic role of aCL antibodies in the induction of thrombosis and fetal loss has been clearly demonstrated in experimental animal models. The effects of  $\text{a}\beta_2\text{Glyp1}$  antibodies on endothelial cell activation, trophoblast cells and induction of fetal loss in experimental animal models also suggest a direct pathogenic role of these antibodies in the development of the antiphospholipid syndrome (APS) [2]. Approximately 30-40% of SLE patients have antiphospholipid antibodies (APL). Antiphospholipid syndrome or antiphospholipid antibody syndrome is an autoimmune, hypercoagulable state caused by antiphospholipid antibodies ( $\text{a}\beta_2\text{Glyp1}$ ). Antiphospholipid syndrome was described after various previous studies estimated specific antibodies in people with systemic lupus erythematosus and thrombosis. Phospholipids such as cardiolipin,  $\beta_2$  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 [3]. 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  $\text{a}\beta_2\text{Glyp1}$ , the cofactor by which aCL binds to phospholipid and usually are pre-sent with aCL [4].

Earlier studies have confirmed that patients' positive for aCA are at risk of repeated epi-sodes of thrombosis, fetal loss and thrombocytopenia [5], 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 [6].

The syndrome is sometimes referred to as "Hughes syndrome", after the rheumatologist Graham R.V. Hughes (St. Thomas' Hospital, London, UK) playing a central role in the description of the condition [7]. APS provokes blood clots (thrombosis) in both arteries and veins as well as pregnancy-related complications such as miscarriage, stillbirth, preterm delivery, or severe preeclampsia [8]. The antiphospholipid syndrome responsible for most of the miscarriages in later trimesters seen in concomitant systemic lupus erythematosus and pregnancy [9]. Patients with both Lupus anticoagulant antibodies and moderate/high titre anticardiolipin antibodies show a greater risk of thrombosis than with one alone.[10] It is advisable to classify APS into one of the following categories for research purposes: I: more than one laboratory criterion present in any combination; IIa: lupus anticoagulant present alone, IIb: anti-cardiolipin IgG and/or IgM present alone in medium or high titers and IIc: anti- $\beta_2$  glycoprotein I IgG and/or IgM present alone in high titers, international research has been created to design and conduct large-scale, multicenter clinical trials in persistently antiphospholipid antibody (aPL) positive patients [11]. In a prospective study of patients with SLE [12] reported, recurrent venous thrombosis, thrombocytopenia, hemolytic anemia, recurrent fetal loss, and leg ulcers are strongly associated with the level of aCL antibody (ACL). The current study was conducted to measure and evaluate the prevalence of aCL and  $\beta_2$ -glycoprotein I autoantibody in Sudanese patients with SLE.

### MATERIALS AND METHODS

This is cross sectional study conducted at Rheumatology clinic at National Ribat University hospital, medical Military hospital, and private clinic in Khartoum State during a period from 2010 to 2012. One hundred and fifteen adult patients with SLE were enrolled (112 were females and 3 were males) age ranged from 18 to 70 with a mean of 34 years. Plasma aCL antibodies and  $\beta$ -2.glycoprotein I antibodies were measured using ELISA technique to evaluate the prevalence of two markers in Sudanese patients with SLE. Cutoff value of aCL is 10 IU/ml, lower positive 10-20 IU/ml, medium positive 20-80 IU/ml and higher positive >80 IU/ml. Cutoff value of  $\alpha\beta$ 2Gly1 is 20 IU/ml, lower positive 20-30 IU/ml, medium positive 30-90 IU/ml and higher positive >90 IU/ml [11].

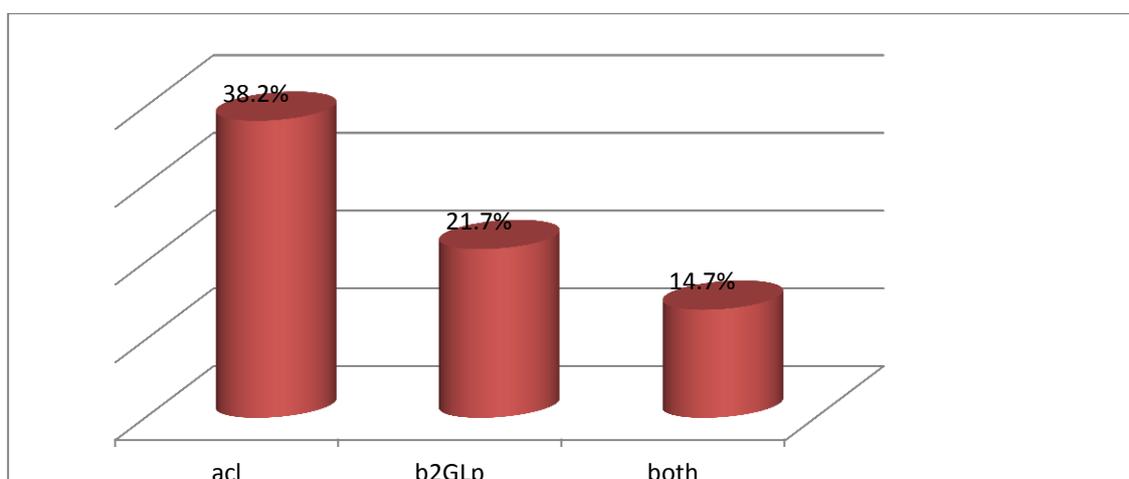
### RESULTS

The prevalence of aCL autoantibody among patients was 44 (38.2 %), 42 (95 %) in females and 5 % ( 2/44) in males. The aCL low titer 23 (52 %), medium titer 18(41 %), and high titer 3 (7.0 %). The prevalence of  $\alpha\beta$ 2Gly1 autoantibody among patients was 25(21.7 %), 24(96 %) in females and 1(4 %) in males. The  $\beta$ 2 GPL low titer 4(16 %), medium titer 14(56%), and high titer 7(28 %) Table 1, both antibodies were determined 14.7% (17/115) of total patients. Figure 1

**Table 1: Prevalence of aCL and  $\alpha\beta$ 2Gly1 antibodies in different SLE among patients with SLE (n = 115).**

Characteristic	aCL	$\alpha\beta$ 2GlyP1
Positive	44/115(38.2%)	25/115 (21.7%)
Female	42/44(95%)	24/25 (96%)
Male	2/44(5%)	1/25 (0.04%)
Low titer	23/44(52%)	4 /25(16%)
Medium titer	18/44(41%)	14/25 (56%)
High titer	3/44(7.0%)	7/25 (28%)

**Figure 1: Shows patients positive for only aCL and  $\alpha\beta$ 2Gly1 antibodies were 38.2 %, 21.7 % respectively, and both antibodies were estimated in 14.7% of total patients.**



acl = anticardiolipdin, b2GLP =  $\beta$ 2glycoprotein, both = acl and b2GLP

## DISCUSSION

Anti-phospholipids' 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. We evaluated the frequencies of aCL and  $\alpha\beta_2$ Glyp1 antibodies in Sudanese patients with SLE, we found the prevalence of aCL auto antibody in patients with SLE was 38.2%, this is in agreement with [11] who reported that the prevalence of anticardiolipin auto antibodies in Iranian SLE patients was 36%, similar results were obtained by [13] revealed aCL and  $\alpha\beta_2$ Glyp1 antibodies among Colombian and Spaniards SLE patients were 40% and 36% respectively. Our findings in agreement with [14] reported that up to 5% to 70% of SLE patients produce some types of aCL.

The prevalence of aCL auto antibodies in SLE Sudanese patients recorded in the current study was higher than the prevalence detected in Turkish SLE patients (27%) [15], and lower than study reported by Anupam *et al.*, 2009 [16] found prevalence of (62%), similar as Mustafa *et al.*, 2010 [17] had reported the prevalence of 16.7% for aCL among SLE patients. Other studies such as Petri., 2010 [18] reported 47% aCL and 32.5%  $\alpha\beta_2$ Glyp1 autoantibodies were determined in SLE. In different populations, the differences of the results may be due to the modality used in the measurement of antibodies, to the study population, ethnic-racial differences and variation of positive criteria with respect to investigators [19].

We found that low titer of aCL 52%, medium titer 41% and high titer 7.0% of total patients, in contrast to Zahra *et al* 2013 low titer was 50.0%, medium 19.4%, and high 30.6% of total patients.

In the present study, the prevalence of anti- $\beta_2$ -glycoprotein I antibody in SLE Sudanese patients was 21.7%. The finding consistent with N. Define *et.al* 2002 [15] estimated that; the frequency of a anti- $\beta_2$ -glycoprotein I antibody was 13%, similar to E Cucurull *et.al* 1999 [13] reported that; the frequency of anti- $\beta_2$ -glycoprotein I antibody was 21% in Colombian and 18% in Spaniards patients with SLE. Our finding inconsistent with study on Turkish patients with SLE found frequency of anti- $\beta_2$ -glycoprotein I antibodies was 83% [20], also no agreement with a study on South African SLE patients, Gould *et al.*, 2006 [21] had reported a very high incidence of 84% for anti- $\beta_2$ -glycoprotein I antibodies where as Inanç M, Radway 1997 [22] had reported an incidence of 33.5% for anti- $\beta_2$ -glycoprotein I antibodies.

We observed that low titer of anti- $\beta_2$ -glycoprotein I antibody was 16%, medium titer 56% and high titer 28% of total patients. It has been reported that the detection of anti- $\beta_2$ -GPI antibodies has a positive association with thrombotic events in patients with primary or secondary APS (with SLE) than the detection of aCL antibodies [23]. Although in our study the prevalence of anti cardiolipin antibodies (38.2%) is more than the prevalence of anti-  $\beta_2$ glycoprotein1 (21.7%). And the prevalence of both antibodies (apl) in our population is 14.7%. Hence detection both aCL and  $\alpha\beta_2$ Glyp1 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 aCL and  $\alpha\beta_2$ Glyp1 antibodies without

thrombotic event is also needed to detect their possible thrombotic event in future along with their clinical presentation.

### CONCLUSION

Our findings, can be concluded that SLE is a disease in which different autoantibody can lead to different patterns of diseases, we found moderate levels of aPLs in Sudanese patients with SLE, particularly, aCLs were found more frequently than  $\alpha\beta_2$ Glyp1antibodies, early detection and management of these antibodies may be useful to minimizing the risk of venous/arterial thrombosis also provide data to design treatment protocols to spare major organs dysfunctions.

### REFERENCES

- [1] Miyakis S, Lockshin M D, Atsumi T, Branch D W, Brey R L and Cervera R. J Thromb Haemostasis 2006;4(2):295-306.
- [2] Sherer Y, Blank M and Shoenfeld Y. Best Pract Res Clin Rheumatol 2007;21(6):1071-1078.
- [3] Zheng H, et al. Arthritis Res Ther 2009;11(3):93.
- [4] Grande J P, Lupus. 1998;7(9):604-610.
- [5] Levine J S, Branch D W and Rauch J. The New England J Med 2002;346:752-763
- [6] Descloux EI, et al. Rheumatol 2008;47(2):183- 187.
- [7] Blank M, Cohen J, Toder V and Shoenfeld Y. Proc Natl Acad Sci USA 1999; 88:3069-3073.
- [8] Gharavi AE, Pierangeli SS and Harris EN. J Autoimmun 2000; 15(2):227-30.
- [9] Haralampos M and Moutsopoulos Panayotis G. Antiphospholipid Antibody Syndrom . Harrison Principles of Medicine. 18 th edition. New York: McGraw-Hill Professional; 2012.
- [10] Svenungsson E, Gustafsson J, et al. Ann Rheum Dis. 2010; 69(5):834-40.
- [11] Zahra Basiri<sup>1</sup>, Mahmoud Gholyaf, Mansureh Faridnia<sup>1</sup>, Ebrahim Nadi, and Mandana Bairanvand. Clinical Acta Medica Iranica 2013; 51(1): 35-40
- [12] Alarcón-Segovia D, Delezé M, Oria CV, Sánchez- Guerrero J, Gómez-Pacheco L Cabiedes J and Fernández LPonce de León S. Medicine 1989; 68(6):353-65.
- [13] Cucurull E, Gharavi A, Diri E, Mendez E, Kapoor D and Espinoza LR. Am J Med Sci 1999; 318: 55 - 60.
- [14] Cervera R, et al. Arthritis Rheum 2002;46(4):1019-27.
- [15] Defne altıntaş N, Olcay tiryaki a, hüseyin tutkak, nurşen düzgülün and GaziTıp Dergisi. Gazi Medical Journal 2008: 19(3): 126-132.
- [16] Anupam Barua, SLE JCMCTA 2009; 20(1):16-22.
- [17] Mostafa A, Ibrahim D H, A. Shehab A and Mohammed AK. J Neuroimmunology 2010;227(1-2):195-201.
- [18] Petri M, Lupus 2010;19(4):419-423.
- [19] Aguirre V, R Cuchacovich, L Barria, H Aris, C Trejo, L Massardo, R Pasten and L Espinoza. Lupus 2001; 10:75-80.
- [20] Mehmet Sahin, Nursen Duzgun, Sevket Ercan Tunca and Huseyin Tutkak. Clin Biochem 2007; 526-530.



- [21] Gould T, Tikly M, Asherson R, Loizou S and Singh S. Scand J Rheumatol 2006;35(1): 29-34.
- [22] Inanç M, Radway-Bright EL and Isenberg DA. Br J Rheumatol 1997; 36:1247–57.
- [23] Vandana P, Anjali R, Pranaya J, Manisha P, Shruti D, Kanjaksha G. Int J Clin Med 2011; 2: 339-345.