DISCUSSION

Systemic lupus erythamatosus disease and antiphospholipid syndrome are important diseases that should not be missed because their burden in young people is large. And their treatment may prevent serious morbidities.[1,43]

Our case-control study was done to show the sensitivity and specificity of antibeta$_2$ glycoprotein IgG and IgM antibodies compared to anticardiolipin antibodies IgG and IgM and lupus anticoagulant antibodies in systemic lupus erythematosus patients with secondary antiphospholipid syndrome.

Our study revealed that:

- Antibeta$_2$ glycoprotein IgM antibody has a sensitivity of 93 % and specificity of 40% in SLE patients with secondary APS, While it has a sensitivity of 90 % and specificity of 40 % in SLE patients without secondary APS. And antibeta$_2$ glycoprotein IgG antibody has a sensitivity of 70 % and specificity of 70% in SLE patients with secondary APS, While it has a sensitivity of 100 % and specificity of 70 % in SLE patients without secondary APS.
- IgM Anticardiolipin antibody has a sensitivity of 20 % and specificity of 90 % in SLE patients with secondary APS, While it has a sensitivity of 15 % and specificity of 100 % in SLE patients without secondary APS. And IgG Anticardiolipin antibody has a sensitivity of 30 % and specificity of 90 % in SLE patients with secondary APS, While it has a sensitivity of 15% and specificity of 90 % in SLE patients without secondary APS.
- So antibeta$_2$ glycoprotein IgM & IgG antibodies are much more sensitive but less specific than anticardiolipin antibodies IgM & IgG in both SLE patients with and without antiphospholipid syndrome.
- Antibeta$_2$ glycoprotein IgM antibodies positively correlate with platelet count in SLE patients with APS.
- Antibeta$_2$ glycoprotein IgM antibodies positively correlate with partial thromboplastin time in SLE patients.

The antibeta$_2$ glycoprotein IgG and IgM antibodies being much more sensitive but less specific than anticariolipin IgG and IgM antibodies in SLE patients with secondary antiphospholipid syndrome these results were in agreement with Parkpian V[1], et al., (2007) study which revealed that:

Antibodies to beta ($_2$)-glycoprotein I (anti-beta($_2$)-GPI) have been reported to have stronger association with clinical antiphospholipid syndrome (APS) than anticardiolipin antibodies (aCL) and lupus anticoagulant (LAC). they investigated the sensitivity and specificity of ELISA for anti-beta($_2$)-GPI in Thai systemic lupus erythematosus (SLE) patients with clinical features of APS and compared the results with IgG/IgM aCL and LAC to find the test with the best association. The hospital records of 151 Thai SLE patients whose sera had been sent for either IgG/IgM anticardiolipin antibodies or lupus anticoagulant testing were reviewed. Sera of patients either without complete clinical records or those with APS-related manifestations other than vascular thrombosis and

pregnancy morbidity (according to the international consensus statement on preliminary classification criteria for definite APS) were excluded. For the remaining subjects (112 patients), their sera were tested for anti-beta($_2$)-GPI antibody, IgG and IgM anticardiolipin, and lupus anticoagulant. The sensitivity and specificity of each method were compared by using the chi-square test. Among the 112 (74.2%) SLE patients in the study, 35 (31.3%) presented with preliminary clinical criteria for APS (i.e., vascular thrombosis and pregnancy morbidity) whereas 77 (68.7%) did not. The sensitivity and specificity of anti-beta($_2$)-GPI determination were 57.1 and 79.2%, respectively, whereas those of IgG aCL were 25.7 and 94.8%, of IgM aCL were 5.7 and 98.7%, and of LAC were 44.8 and 77.3%, respectively. The accuracy of the four tests showed similar association with clinical APS (accuracy of test = 72.3, 73.2, 69.6, and 68.3%, respectively). Concerning the sensitivity, specificity, and difficulty of the methods, the combination of anti-beta($_2$)-GPI and IgG aCL tests was the best for the diagnosis of APS in Thai SLE patients.

Another study by Danowski A[1], et al., (2006): adopted the research of Anti-beta$_2$-glycoprotein I: prevalence, clinical correlations, and importance of persistent positivity in patients with antiphospholipid syndrome and systemic lupus erythematosus in comparison to anticardiolipin (aCL) and the lupus anticoagulant (LAC). They investigated whether serial samples improve clinical utility.

Serum samples for anti-beta$_2$-GPI (IgG, IgM, IgA), aCL (IgG, IgM, IgA), and LAC (by dilute Russell viper venom time; RVVT) were collected from 418 consecutive patients with SLE or APS between October 2002 and March 2003. Clinical and serologic data of these patients were analyzed. And the results were:

A total of 185 (44.5%) patients were positive for anti-beta$_2$-GPI, 55.3% were positive for aCL, and 31.1% for LAC. Anti-beta2-GPI was more common in Caucasians than in African Americans ($p = 0.098$). IgM and IgA were the most frequent isotypes of anti-beta$_2$-GPI. aCL and anti-beta$_2$-GPI were highly associated ($p < 0.0001$ to $p = 0.0177$, depending on isotype). A positive association was found between the presence of the LAC by dilute RVVT and anti-beta$_2$-GPI IgG ($p < 0.0001$), IgM ($p < 0.0001$), and IgA ($p = 0.0002$) antibodies. Persistent positivity increased the association of venous and arterial thrombosis with anti-beta$_2$-GPI (IgG and IgM isotypes). Pregnancy loss, seizures, and migraines were not associated with anti-beta$_2$-GPI. IgA anti-beta$_2$-GPI was not significantly associated with any manifestation of APS.

And the conclusion was that:

The prevalence of anti-beta$_2$-GPI IgM and IgA was very high. Measurement of anti-beta$_2$-GPI IgG is clinically useful in identifying patients with SLE at higher risk for venous and arterial thrombosis. Persistent positivity increased the association of IgG anti-beta$_2$-GPI with venous thrombosis and anti-beta$_2$-GPI IgM with arterial thrombosis. IgA anti-beta$_2$-GPI was not significantly associated with APS manifestations.

But our study in at the point of that there is a higher sensitivity but lower specificity of antibeta$_2$ glycoprotein IgM and IgG than anticardiolipin IgM and IgG in SLE patients with and without secondary APS antibodies was in contrast to Day HM[1], et al., (1998) study that showed: Frequencies, sensitivities, specificities, and predictive values and correlations of anti-beta$_2$-GPI which were compared to the aPL ELISA (IgG and IgM) and LAC for individual (and combined) features of APS. IgG and IgM anti-beta$_2$-GPI

antibodies were determined by ELISA in 281 patients with SLE, primary APS, or other connective tissue diseases and the results were:

Among 139 patients with positive aPL ELISA and/or LAC tests, 57 (41%) had anti-beta$_2$-GPI antibodies (IgG and/or IgM) compared to 11% of patients with SLE negative for these tests (p = 0.00001). In 130 patients with APS, anti-beta$_2$-GPI occurred in 42% and tended to be more specific but less sensitive than the aPL ELISA or LAC. When all 3 aPL tests were combined, the best sensitivities and negative predictive values were achieved; however, specificity and positive predictive values remained low. Anti-beta$_2$-GPI antibodies occurred more frequently in primary APS (58%) vs secondary antiphospholipid syndromes (33%) (p = 0.008, OR = 2.9). Among 79 patients with SLE negative by both aPL ELISA and LAC, 9 (11 %) were positive for anti-beta$_2$-GPI, 7 of whom had clinical features consistent with APS (representing 5% of all with APS). Stepwise multiple logistic regression analysis revealed beta$_2$-GPI to be most strongly associated with neurological syndromes other than stroke, deep venous thrombosis, and recurrent fetal loss, while LAC was most strongly correlated with stroke and thrombocytopenia. IgM aPL antibodies also were independently associated with neurological syndromes and recurrent fetal loss.

And their conclusion was:

Testing for beta$_2$-GPI antibodies may be clinically useful in the diagnosis of APS but cannot supplant other aPL ELISA or LAC. Multivariate analyses suggest that anti-beta$_2$-GPI antibodies may play a more central role in certain clinical manifestations of APS than antibodies detected by the aPL ELISA or LAC.

Also in contrast to our study ZOHOURY, NAVID BSc (2013) study stated that:

In the entire cohort, anti-β_2GPI-D1 antibodies differentiated APS and controls with a sensitivity and specificity of 49.8% and 99.6%, respectively. The likelihood ratios were LR+ 136.5 and LR- 0.5. The prevalence of anti-β_2GPI antibodies was higher in primary than in secondary APS and reach significance for anti-β_2GPI-D1 (p=0.0029), but not for anti-β_2GPI antibodies (p=0.06). When compared with the anti-β_2GPI assay, both assays showed good qualitative (79.8%, 95% CI 69.1-86.5; *kappa*=0.60, 95% CI 0.44-0.76) and quantitative agreements (spearman`s *rho*=0.81, 95% CI 0.73-0.87) as well as similar discrimination between APS patients and controls as shown by ROC analysis. In the smaller sub-cohort (n=622), sensitivity/specificity were 50.2%/99.2% (β_2GPI-D1) and 72.8%/87.3% (β_2GPI), respectively.

When APS patients were stratified according to the history of thrombosis, anti-β_2GPI-D1 and anti-β_2GPI antibodies showed sensitivities/specificities of 37.9%/93.5% and 60.3%/67.7% respectively. At high specificity (96.8%), the sensitivity for thrombosis was significantly higher for β_2GPI-D1 than for β_2GPI (22.4% vs. 12.1%; $p<0.05$).

And the Conclusion was that:

Anti-β_2GPI-D1 and anti-β_2GPI antibodies show similar performance characteristics in differentiation of APS patients and controls. The correlation with history of thrombosis appears to be stronger for anti-β_2GPI-D1 compared to anti-β_2GPI-antibodies. Therefore, anti-β_2GPI-D1 antibodies are a promising biomarker to aid in the diagnosis and risk assessment of APS patients, but further studies are needed to verify those preliminary

findings. This opposed the idea of usefulness of antibeta$_2$ glycoprotein in diagnosing APS syndrome that was introduced in our study.

The finding in our study that there is a positive significant correlation between IgM antibeta$_2$ antibodies in SLE patients with secondary APS and platelet count was against the studies: G Lakos, et al., dealing with Relationships between IgG, IgA and IgM anti-β_2-GPI antibodies and the symptoms of APS

The association of anti-β_2-GPI antibodies of all isotypes with arterial or venous thrombosis, thrombocytopenia, spontaneous fetal loss, livedo reticularis, epilepsy, heart valve disease and the presence of LA were calculated . Increased amounts of IgM anti-β_2-GPI antibodies were related only to thrombocytopenia (P = 0.04) and heart valve disease (P = 0.01).IgG anti-β_2-GPI antibody levels were significantly higher in patients with previous venous thrombosis (P = 0.003), thrombocytopenia (P = 0.01), intra-uterine fetal loss (P = 0.01) and heart valve disease (P = 0.03). Elevated IgG antibody levels were measured also in patients with arterial thrombosis, close to significance (P = 0.06). IgA anti-β_2-GPI antibody values were significantly increased in patients with venous thrombosis (P = 0.007) and thrombocytopenia (P = 0.02), and also in patients with livedo reticularis (P = 0.01), heart valve disease (P = 0.02) and epilepsy (P = 0.01). Moreover, nearly significant elevation was detected in patients with arterial thrombosis (P = 0.06).

Also in contrast to our study Bourhim M[1], et al., study stated that the Anti-beta$_2$-glycoprotein I antibodies recognizing platelet factor 4-heparin complex in antiphospholipid syndrome in patient substantiated with mouse model. The antiphospholipid syndrome is defined by the presence of antiphospholipid antibodies associated with arterial and/or venous thrombosis, and recurrent abortion accompanied often by thrombocytopenia. These antibodies are heterogeneous and react against phospholipid-binding proteins such as beta$_2$-glycoprotein I (beta$_2$GPI) and prothrombin. The recognition of anti-beta$_2$-glycoprotein I (anti-beta$_2$GPI) by platelet factor 4-heparin complex (PF4-Hc) has been previously evoked and partially confirmed by the present inhibition studies. Further, the anti-beta$_2$-glycoprotein I antibodies were purified from a patient with primary antiphospholipid syndrome using Affi-gel-10-beta$_2$GPI immunoaffinity chromatography. The purified anti-beta$_2$GPI IgM as well as patient serum equally recognized PF4-Hc in ELISA mode. In order to substantiate this data and to better understand we studied an animal model using mouse active immunization with the purified human anti-beta$_2$GPI. The mice showed a significant decrease in their platelet count. In addition the ELISA responses of the immunized mice sera were positive against both beta$_2$GPI and PF4-Hc, substantiating the double recognition. Despite many previous reported animal model studies, this is the first time they have shown the specific recognition of anti-beta$_2$GPI antibodies by PF4-Hc, the results in the induced mice correlating the data observed with some patients.

The finding in our study that showed a positive significant correlation between antibeta$_2$ glycoprotein IgM antibodies and partial thromboplastin time found in SLE patients agreed with the study Clinical significance of anticardiolipin and anti-beta$_2$-glycoprotein I antibodies by Obermoser G, et al., When ACl were tested in 3,600 consecutive sera in our laboratory between January 1999 and June 2001. The clinical diagnosis and prevalence of thrombosis and pregnancy morbidity were retrospectively reviewed in aCl-positive patients. Furthermore, the frequency of anti-beta$_2$-gpI antibodies,

lupus anticoagulant (LA), prolonged activated partial thromboplastin time (aPTT), and thrombocytopenia were investigated in aCl-positive patients.

And results were:

147 aCl-positive patients, 110 women and 37 men with a mean age of 41 years (range 7.8-82.5), were identified. 42 (28.6%) aCl-positive patients fulfilled the criteria for APS which was secondary to a connective tissue disorder in 8 patients. The frequency of anti-beta$_2$-gpI antibodies and LA, prolonged aPTT, and thrombocytopenia in aCl-positive patients was 23.8, 27.2, 25.7 and 9.2%, respectively. The presence of both aCl and anti-beta$_2$-gpI antibodies was strongly associated with clinical symptoms of APS ($p = 0.007$) compared to $p = 0.008$ for LA.

And the conclusion was:

Their data suggest that assessment of anti-beta$_2$-gpI antibodies in addition to aCl is a valuable diagnostic tool in the workup of patients with APS.

We can conclude from our study that:

IgM and IgG antibeta$_2$ glycoprotein being highly sensitive in detecting APS can be used for its diagnosis but prefered to be combined with anticardiolipin antibodies IgM and IgG due to its higher specificity in APS.

Also our study showed that when IgM antibeta$_2$glycoprotein antibodies increase, platlet count increases in SLE patients with secondary APS; and the explanation of this finding; thrombocytosis with increase of antibeta$_2$glycoprotein IgM in SLE with secondary APS; may be due to:

Thrombocytosis may be a cause of acute thrombosis, so upcoming researches should adopt studying this relation because this finding may be existing inspite of thrombocytopenia found as a clinical picture and a sign of activity of APS in some patients.

Platlets may clump into rafts leaving the slide under microscopic examination apparently devoid of platlets in old (unfresh) blood samples so slides should be scanned for rafts before reporting platlet number, also it may be attributed to increased platlet aggregation in APS that platlet count under microscope appears decreased; this was for the technical reasons.

The previous reasons are supporting the result of our study that platlet count increases in SLE patients with secondary APS.

Or increase or decrease in platlet count may be owing to a cause other than APS; examples of conditions causing increase in platlet count: myeloproliferative disorders and/or polycythemia vera, and less commonly with infections, blood loss, and splenectomy; rare causes of ↑ PC include anemia–hemolytic, iron-deficiency, sickle cell, cirrhosis, collagen vascular disease, cryoglobulinemia, drugs-epinephrine, OCs, exercise, hemorrhage, hypoxia, ITP, post-partum, pregnancy, rheumatoid arthritis, TB. While conditions causing decrease in platlet count: increase in bleeding tendency; platelets are ↓ in malignacies of bone, GI tract, brain, leukemia, kidney or liver disease, aplastic anemia,

DIC, ITP, SLE, drugs associated with decrease platelet count: aspirin, chemotherapeutic agents, chloromycetin, phenylbutazone, quinidine, thiazide diuretics, tolbutamide.

Or the disease from the beginning is mis-diagnosed as APS but it is another cause of vascular thrombosis or pregnancy morbidity.

Also we concluded that IgM antibeta$_2$ glycoprotein when increase the partial thromboplastin time in SLE patients without secondary APS become prolonged. This may be due to the role of phospholipid in phospholipid-dependent clotting assay.

SUMMARY

Systemic lupus erythematosus (SLE) is the prototypic multisystem autoimmune disorder with a broad spectrum of clinical presentations encompassing almost all organs and tissues. Morbidity can be fatal—if not treated early—in some patients.

Antiphospholipid syndrome (APS) is a disorder that manifests clinically as recurrent venous or arterial thrombosis and/or fetal loss. It may be primary or secondary to other associating condition.

Antiphospholipid antibodies are immunoglobulins, belonging to a group of heterogeneous autoantibodies directed against phospholipids and phospholipid protein complexes. The presence of these immunoglobulins has been associated with thromboembolic events such as deep vein thrombosis, pulmonary embolism, myocardial infarction and stroke and pregnancy morbidity.

The aim of the work was to investigate the specificity and sensitivity of antibeta$_2$ glycoprotein (anti-β_2 GPI) IgG/IgM by ELISA in Egyptian SLE patients with features of APS compared to IgG/IgM anticardiolipin (aCL) and lupus anticoagulant (LAC), and to examine the possible correlations between clinical manifestations and anti- β_2 GPI levels.

The present study was carried out on three groups of young subjects:

Group "I": Thirty systemic lupus erythematosus patients having secondary antiphospholipid syndrome. (28 females, 2 males aged from 21-54 years old)

Group "II": Twenty systemic lupus erythematosus patients without antiphospholipid syndrome. (19 females, one male aged from 18-46 years old)

Group "III": Twenty young healthy subjects served the purpose of control. (18 females, 2 males aged from 20-44 years old)

All studied groups were subjected to the following:

- Recording of historical data for patients including personal, present, past, family, drug and obstetrical, menstrual history, previous laboratorical and radiological investigations.
- Thorough clinical examination.
- Laboratory assessment including: ANA , antidsDNA ,CBC, PT , aPTT, serum urea , serum creatinine , urine analysis, albumin/creatinine ratio in urine or 24 hours urinary proteins , lupus anticoagulant and (ELISA) technique which was used for the detection of antibeta2 glycoprotein IgM, IgG and anticardiolipin IgM and IgG antibodies.

The results showed that serum antibeta$_2$ glycoprotein IgM antibody has a sensitivity of 93 % and specificity of 40% in SLE patients with secondary APS, While sensitivity of 90 % and specificity of 40 % in SLE patients without secondary APS.

Antibeta$_2$ glycoprotein IgG antibody has a sensitivity of 70 % and specificity of 70% in SLE patients with secondary APS syndrome, while it has a sensitivity of 100 % and specificity of 70 % in SLE patients without secondary APS.

IgM anticardiolipin antibody has a sensitivity of 20 % and specificity of 90 % in SLE patients with secondary APS syndrome, while it has a sensitivity of 15 % and specificity of 100 % in SLE patients without secondary APS.

IgG anticardiolipin antibody has a sensitivity of 30 % and specificity of 90 % in SLE patients with secondary APS syndrome, While it has a sensitivity of 15% and specificity of 90 % in SLE patients without secondary APS.

Antibeta$_2$ glycoprotein IgM positively and significantly correlates with platelet count in SLE patients with APS and this finding should be investigated in upcoming researches.

Antibeta$_2$ glycoprotein IgM positively and significantly correlates with partial thromboplastin time in SLE patients.

There was a positive but non significant correlation between IgM and IgG antibeta$_2$ glycoprotein and lupus anticoagulant in SLE patients with and without secondary APS.

It was concluded from the current study that antibeta2 glycoprotein IgM and IgG antibodies can be used in diagnosing SLE with secondary APS but it is not highly specific to APS. Anticardiolipin IgM and IgG antibodies although more specific than antibeta2 glycoprotein IgM and IgG antibodies, they can miss cases of APS due to their lower sensitivity. Platelet count increases with the increase in antibeta$_2$ glycoprotein IgM antibodies in SLE patients with secondary APS. And aPTT increases with the increase of antibeta$_2$ glycoprotein IgM antibodies in SLE patients.

CONCLUSION

1- Antibeta$_2$ glycoprotein IgM antibody has a sensitivity of 93 % and specificity of 40% in SLE patients with secondary APS, while it has a sensitivity of 90 % and specificity of 40 % in SLE patients without secondary APS.

2- Antibeta$_2$ glycoprotein IgG antibody has a sensitivity of 70 % and specificity of 70% in SLE patients with secondary APS, while it has a sensitivity of 100 % and specificity of 70 % in SLE patients without secondary APS.

3- IgM Anticardiolipin antibody has a sensitivity of 20 % and specificity of 90 % in SLE patients with secondary APS, while it has a sensitivity of 15 % and specificity of 100 % in SLE patients without secondary APS.

4- IgG Anticardiolipin antibody has a sensitivity of 30 % and specificity of 90 % in SLE patients with secondary APS, While it has a sensitivity of 15% and specificity of 90 % in SLE patients without secondary APS.

5- So antibeta$_2$ glycoprotein IgM & IgG antibodies are much more sensitive but less specific than anticardiolipin antibodies IgM & IgG in both SLE patients with and without antiphospholipid syndrome.

6- There is a positive significant correlation between antibeta$_2$ glycoprotein IgM positively correlates and platelet count in SLE patients with APS, i.e. platelet count increases with the increase in antibeta$_2$ glycoprotein IgM antibodies in SLE patients with secondary APS.

7- There is a positive significant correlation between antibeta$_2$ glycoprotein IgM and partial thromboplastin time in SLE patients, i.e. aPTT increases with the increase of antibeta$_2$ glycoprotein IgM antibodies in SLE patients.

8- There is a positive but non significant correlation between IgM and IgG antibeta$_2$ glycoprotein and lupus anticoagulant in SLE patients with and without secondary APS.