Biological Value of Annexin AV and Anti-annexin Antibodies in patients with systemic lupus erythematosus and Anti-phospholipid (Hughes) syndrome

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Abstract: Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease. Anti-phospholipid (APL) antibodies arc frequently found in SLE patients. Fifty percent of Anti-phospholipid syndrome(APS) is secondary to SLE. Annexin AV (ANX AV) exhibits anti-coagulant properties, but aPL antibodies formed against it interfere with its function. The study aimed to investigate whether anti-annexin AV (aANX AV) antibodies (lgM/IgG) or ANX AV plasma level is related to pathophysiology of SLEor APS. Measurement of aANX AVantibodies (lgM/G) and ANX AV plasma level by Enzyme Linked Immunosorbent Assay (ELISA) in 40 patients divided into two groups SLE and APS and 20 healthy control of matched age. Anti-ANX AV lgM antibodies were significantly associated with APS; this was revealed when we compared patients with Primary APS and Secondary APS to the control group. There was significant difference in ANX AV plasma level when compared SLE to Primary APS and Secondary to Primary APS. ANX AV plasma levels were not affected by the presence of Anti ANV lg M/G. Conclusively, it could be suggested that the ANX AV plasma level may contribute to susceptibility (low levels) or the protection (high levels) against hypercoagulability in APS patient. It may also reflect the severity of SLE. In addition, ANX AV plasma levels are not affected by the presence of Anti ANV lgM/G. ANX AV plasma levels may be an import tool in diagnosis and also target for treatment.

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Key words: Systemic lupus erythematosus, Anti-phospholipid syndrome, Anti-phospholipid antibodies, Anti-ANX AV antibodies (lgM/IgG), ANX AV plasma level.

1. Introduction

Annexin AV (or annexin- A5) a phospholipid-binding protein that belongs to the annexin family, it binds to negatively charged phospholipids on lipid bilayers in a calcium dependent manner and possesses a high affinity for phosphatidyl serine (PS). Such arrangement affects membrane properties including rigidity, fluidity and lipid segregation. It participates in the regulation and stabilization of membrane domains and promote cell membrane repair.ANX AV is found in many tissues including blood. Once it is in the plasma, it binds to blood cells (platelets and erythrocyte) or to endothelial cells (Bouter *et al.,* 2011).As a part of normal haemostasis, anionic phospholipids on the surface of the cell membrane bilayer serve as potent cofactors for the assembly of three coagulation complexes: the tissue factor (TF)-VIIa complex, the IXa- VIIa complex and the Xa-Va complex. The presence of phospholipid accelerates blood coagulation (Adams and Bird, 2009).

Annexin AV, in absence of APL-antibodies, forms two-dimensional crystals, which bind with high affinity to the anionic phospholipid surface creating a sort of "anticoagulant shield" which blocks the assembly of the phospholipid-dependent coagulation complexes and inhibits coagulation. APL-antibodies disrupt the ability of ANX AV to cluster on phospholipid surface. As a result, coagulation is accelerated and the development of thrombosis is promoted (Irman *et al.,* 2009).ANX AV participates in the membrane repair process by binding to PS molecules exposed at the edges of torn membranes thereby helping in membrane fusion events. During apoptosis, binding of extra-cellular ANX AV to cell surface-exposed PS may prevent the occurrence of coagulation and inflammation (Blankenberg, 2009). ANX AV has also systemic anti-inflammatory properties (Ewing *et al.,* 2011).

Anti-ANX AV immunoglobulin G or M (lgG /IgM) antibodies are directed against ANX AV, may contribute in pathophysiology of Antiphospholipid Syndrome (APS) and Systemic lupus erythematosus (SLE) (Hrycek and Cieslik, 2011).

SLE is a chronic inflammatory autoimmune disease characterized by production of autoantibodies and formation of immune complex (Alessandri *et al.,* 2011).SLE affects mainly young women with a peak incidence between the ages of 15 and 40years. Female

to male ratio10:1(Pons-Estel *et al.*, 2010). Multiple autoantibodies induce tissue damage either by binding directly to self antigen or inducing inflammation following the tissue deposition of immune complex which occurs in many tissues including glomeruli, skin and lungs leads to manifestations of the disease (Ahmed and Auolik, 2010). Anti-phospholipid antibodies (APL) are frequently found in patients with SLE. The term "Antiphospholipid Syndrome or Hughes Syndrome" denote to the clinical association between APL antibodies and a syndrome of hypercoagulability, which is an acquired autoimmune disorder characterized by arterial and/or venous thrombosis and circulating APL antibodies (Li *et al.*, 2011). APS can be primary or secondary to other autoimmune diseases, 50% of APS is secondary to SLE. APL antibodies are autoantibodies directed against phospholipids and phospholipid binding plasma proteins (Meroni *et al.*, 2011).

Aim of study:

To find out a relation between Anti-ANX AV antibodies (IgG/M) or ANX AV plasma level and the pathophysiology of SLE and APS.

2. Subjects and Methods

This study included 60 subjects aged between 20 to 40 years old from Al-Zaharaa Hospital in Cairo and divided into 2 groups: SLE, APS (Primary and secondary to SLE). Twenty healthy females of matched age served as control group. All groups were subjected to full history, complete medical examination and laboratory investigations (Complete blood count, erythrocytes sedimentation rate and autoimmune profile-C3 and C4 levels, lupus anticoagulant test and anticardiolipin (aCL) antibodies). Measuring of Annexin AV plasma level and Anti-Annexin AV antibodies (lgG&IgM) by Enzyme Linked Immunosorbent Assay (ELISA) technique for the quantitative determination of lgG and IgM autoantibodies to AnnexinV. ELISA were done using kits purchased from (Orgentic Diagnostika GmbH) 55129 Mainz-Germany.

Statistical analysis:

Data was analyzed using statistical package for social science (SPSS) version15.Numerical data were expressed as mean ± standard deviation (SD) and range as appropriate. Probability value (*P*-value) less than 0.05was considered significant and highly significant less than 0.01.

3. Results

Statistical comparison between SLE and Control groups as regards Annexin AV(ANX AV) plasma level and Anti- Annexin AV (Anti-ANX AV) antibodies (lgG&IgM) revealed, no significant association was found between the presence of ANX AV plasma level and Anti- ANX AV antibodies (lgG&IgM) in SLE patients as compared to control groups (Table 1 and Figure 1).

Table (1): Comparison between SLE and control groups as regards ANX AV plasma level and Anti-ANX AVantibodies (lgG&IgM)

	ANX- AV (ng/ml)	Anti-ANX-AV-lgG (Au/ml)	Anti-ANX AV-lg M (Au/ml)
SLE	**15.14±7.04**	**5.21±2.35**	**7.30±4.85**
Control	**12.93±7.45**	**5.00±1.88**	**5.15±2.01**
P-value	**0.31**	**0.73**	**0.07**

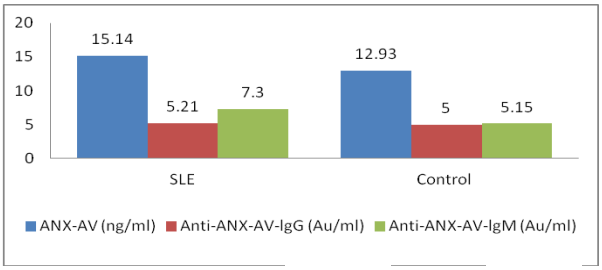

Figure (1): Mean levels of ANX AV andAnti-ANX AV antibodies (lgG&IgM) in SLE and control groups

Statistical comparison between primary APS and Control groups as regards ANX AV plasma level and Anti-ANX AV antibodies (lgG&IgM) revealed no significant association was found between the presence of ANX AV plasma level in primary APS patients as compared to control groups (Table 2 and Figure 2). There was a significant association between the presence of Anti-ANX AV IgM and primary APS (*P* value ≤ 0.05) as compared to control group (Table 2 and Figure 2).

Table 2: Comparison between Primary APS and control groups as regards ANX AV plasma level and Anti-ANX AV antibodies (lgG & IgM)

	ANX-AV(ng/ml)	Anti-ANX-AV-lgG(Au/ml)	Anti-ANX AV-lg M(Au/ml)
Primary APS	**8.40±8.55**	**4.82±1.60**	**33.07±54.35**
Control	**12.93±7.45**	**5.00±1.88**	**5.15±2.01**
P-value	**0.19**	**0.83**	**0.02***

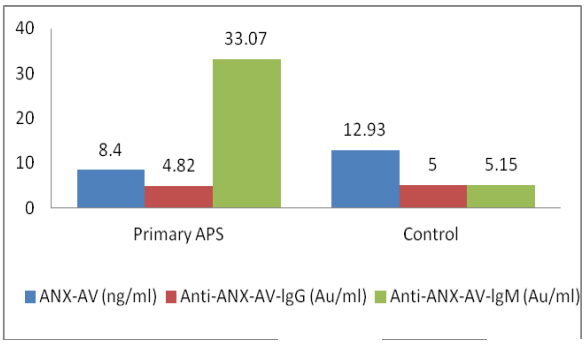

Figure (2): Mean levels of ANX AV and Anti-ANX AV antibodies (lgG&IgM) in Primary APS and control groups

Statistical comparison between secondary APS and Control groups as regards ANX AV plasma level and Anti-ANX AV antibodies (lgG & IgM) revealed no significant association between the levels of ANX AV in secondary APS patients as compared to control group (Table 3 and Figure 3). There was a significant (borderline) association between the presence of Anti-ANX AV IgM and secondary APS (*P* value: 0.05) as compared to control group (Table 3 and Figure 3).

Table (3): Comparison between Secondary APS and control groups as regards ANX AV plasma level and Anti-ANX AV antibodies (lgG & IgM)

	ANX-AV(ng/ml)	Anti-ANX-AV-lgG(Au/ml)	Anti-ANX AV-lg M(Au/ml)
Secondary APS	**17.50±6.65**	**9.10±14.39**	**7.70±5.8**
Control	**12.93±7.45**	**5.00±1.88**	**5.15±2.01**
P-value	**0.07**	**0.21**	**0.05***

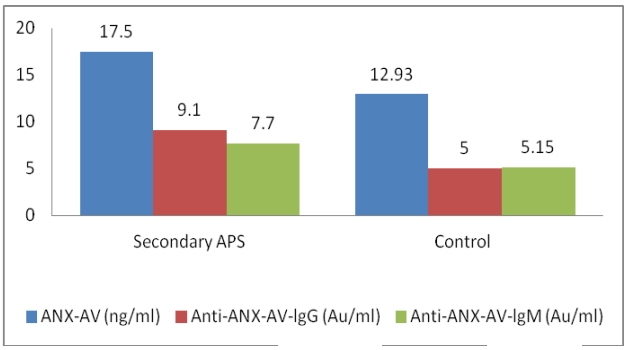

Figure (3): Mean levels of ANX AV and Anti-ANX AV antibodies (lgG&IgM) in Secondary APS and control groups

Comparison between groups of patients regarding ANX AV plasma level and Anti-ANX AV antibodies (lgG & IgM) revealed to a significant difference in ANX AV plasma level (*P* value: 0.04) which was higher in SLE as compared to primary APS patients and in Anti-ANX AV IgM (*P* value: 0.02) which was higher in primary APS (Table 4 and Figure 4), but no differences exist between SLE and secondary APS (Table 5 and Figure 4).Comparing primary to secondary APS patients, there was a highly significant difference in ANX AV plasma level in secondary APS (*P* value ≤ 0.01) (Table 6 and Figure 4).

Table (4): Comparison between SLE and Primary APS patients as regards ANX AV plasma level and Anti-ANX AV antibodies (lgG&IgM)

	ANX-AV(ng/ml)	Anti-ANX-AV-lgG(Au/ml)	Anti-ANX AV-lg M(Au/ml)
SLE	**15.14±7.04**	**5.21±2.35**	**7.30±4.85**
Primary APS	**8.40±8.55**	**4.82±1.60**	**33.07±54.35**
P-value	**0.04***	**0.68**	**0.02***

Table (5): Comparison between SLE and Secondary APS patients as regards ANX AV plasma level and Anti-ANX AV antibodies (lgG & IgM)

	ANX-AV(ng/ml)	Anti-ANX-AV-lgG(Au/ml)	Anti-ANX AV-lg M(Au/ml)
SLE	**15.14±7.04**	**5.21±2.35**	**7.30±4.85**
Secondary APS	**17.50±6.65**	**9.10±14.39**	**7.70±5.8**
P-value	**0.30**	**0.19**	**0.80**

Table (6): Comparison between Primary and Secondary APS patients as regards ANX AV plasma level and Anti-ANX AV antibodies (lgG & IgM)

	ANX- AV (ng/ml)	Anti-ANX-AV-lgG(Au/ml)	Anti-ANX AV-lg M(Au/ml)
Primary APS	**8.40±8.55**	**4.82±1.60**	**33.07±54.35**
Secondary APS	**17.50±6.65**	**9.10±14.39**	**7.70±5.8**
P-value	**0.01**	**0.19**	**0.80**

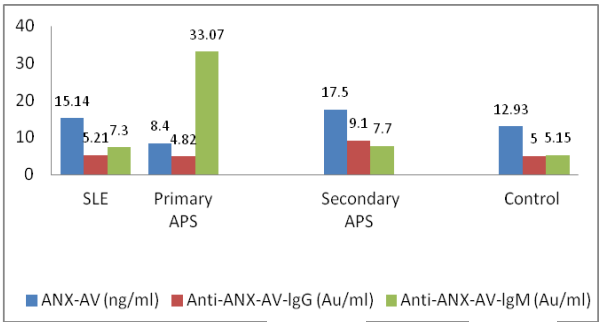

Figure (4): Mean levels of ANX AV and Anti-ANX *AV antibodies (lgG&IgM) in all groups*

On correlating between patients receiving treatment and Annexin AV plasma level and Anti-Annexin antibodies (lgG & IgM) ,there were significant correlations between patients receiving treatment and Annexin AV plasma level and Anti-Annexin AV IgM, but no relation exists with Anti-Annexin AV IgG (Table 7).

Table (7): Correlation between patients receiving treatment and Annexin AV plasma level and Anti- Annexin AV antibodies (lgG & IgM)

	ANX- AV(ng/ml)	Anti-ANX-AV-lgG(Au/ml)	Anti-ANX AV-lg M(Au/ml)
P-value	**0.01***	**0.19**	**0.01***

4-Discussion

SLE is a chronic inflammatory autoimmune disease characterized by production of auto-antibodies &formation of immune complex. These auto-antibodies are produced due to abnormalities of B and T cells ranging from loss of tolerance to self antigens to hyperactive B cell and aberrant T cell regulation which leads to autoimmunity (Crispin *et al.*, 2010).

The hallmark of SLE is the production of auto-antibodies. Anti-phospholipid antibodies (APL) are frequently found in patients with SLE. Annexine (ANX AV) is an antigenic target of a PL antibodies and may be involved in pathophysiology of disease, ANX AV forms two-dimensional crystal that binds with high affinity to the surface of anionic phospholipids creating a sort of "anticoagulant shield". This blocks the assembly of the phospholipids-dependent coagulation complexes, thereby inhibiting coagulation (Woo *et al.*, 2010).

In the present study, a significant association between Anti-ANX AV IgM and APS was found. This was revealed with comparing patients with Primary APS and Secondary APS to the control group(*P* value≤0.02 and 0.05 respectively).The Anti-ANX AV IgM was significantly higher in Primary APS than in SLE**(** *P* value≤ 0.02).But, no significant difference between groups of patients (SLE and APS) and control group regarding Anti-ANX AV Ig G was found.

This finding was in accordance with the result of, De Laat *et al.*, (2006) they found that APS was significantly associated with elevated level of Anti-ANX AV antibodies compared with that for healthy control subject. Also Shoenfeld *et al.*, (2009) reported that the Anti-ANX AV antibodies among patients with APS were found to be significantly higher than controls and Anti-ANX AV antibodies are rarely found in SLE, but their presence was more frequent in patients with secondary APS.

In this study, it is found a significant association between Anti-ANX AV antibodies and APS; this was supported by Alessandri *et al.*, (2011) who reported that aPL antibodies directed towards ANX AV may cause thrombosis and these molecules may allow improving the knowledge of the pathogenesis and the early identification of APS.

As it was reported in this study, there was significant difference in ANX AV level when compared SLE to Primary APS (*P* value: 0.04) and Secondary to Primary APS (*P* value: 0.01**),** but there was no difference when we compared ANX AV plasma levels between groups of patients (SLE and APS) and control group. Concomitant with our result, Hrycek and Cieslik, (2011) found that ANX AV plasma level was significantly higher in SLE patients when compared to other groups. They explained this elevation of ANX AV level by

disturbances in the process of clearance of apoptotic cells occurring in SLE. Apoptotic cells display PS which leads to accumulation and binding of extracellular ANX AV.

In this study, we found that ANX AV plasma levels were not affected the presence of Anti-ANX AV (Ig G/ M)(P value:0.09 and 0.07 respectively).This finding was in accordance with the result of**,** De Laat *et al.,* (2006) didn't find a correlation between ANX AV plasma level with Anti-ANX AV (Ig G, Ig M or both).

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