Sensitivity and Specificity of Immunoglobulin A Isotype of Anti-β2 Glycoprotein I in Patients with Systemic Lupus Erythematosus and Secondary Antiphospholipid Syndrome

AZZA H. EL-AWAR, M.D.*; TAMER M.A. GHEITA, M.D.*; DOAA H. SAYED, M.D.*; MAGDA I.M. AYOUB, M.D.** and ASMAA M. ABD-ALAAL, M.D.***

The Departments of Rheumatology & Rehabilitation*, Microbiology & Immunology** and Chemical Pathology***, Faculty of Medicine, Cairo University, Cairo, Egypt

Abstract

Introduction: Systemic lupus erythematosus (SLE) is an autoimmune disease of chronic course characterized by the presence of autoantibodies to the cell nucleus. Antiphospholipid syndrome (APS) is considered the most common cause of acquired thrombophilias. The diagnosis of APS is made on the presence of clinical manifestations of thrombosis/pregnancy morbidity as well as persistently positive antiphospholipid antibodies (aPL) of any type i.e. anticardiolipin (aCL) IgM/IgG or anti-β2 glycoprotein I (anti-β2GPI) (IgM/IgG) or lupus anticoagulant (LA).

Aim of the Work: Identifying the sensitivity and specificity of anti-β2-GPI of IgA isotype among Egyptian SLE patients having and lacking APS.

Patients and Methods: The study was done on 54 SLE patients. Twenty seven (50%) of patients had no APS while others had secondary APS. Patients having other known forms of thrombophilia were excluded. Twenty seven apparently normal control persons of matched age and sex were included. Clinical assessment and routine laboratory tests were done. Patients and controls were assessed for positivity of lupus anticoagulant, anti-β2GPI of IgM, IgG and IgA isotype in addition to aCL of IgM, IgG and IgA isotypes.

Results: IgA isotype of anti-β2GPI antibodies had a low specificity but high sensitivity in Egyptian lupus patients.

Conclusion: Anti-β2GPI of IgA isotype could be considered in assessing lupus patients for APS when all standardized aPL are negative owing to its high sensitivity.


Introduction

SYSTEMIC lupus erythematosus (SLE) is an autoimmune disease of chronic course of remissions and exacerbations. It is characterized by the presence of autoantibodies to cell nucleus. Almost all organ systems could be affected producing protean clinical manifestations [1].

Antiphospholipid syndrome (APS) is considered the most common form of thrombophilias [2]. Stroke is considered the most common manifestation of arterial thrombosis while deep venous thrombosis is considered the most frequent form of venous thrombosis is. Pregnancy losses in those patients typically occur after the 10th week of pregnancy. The diagnosis of APS is made on the presence of clinical manifestations as well as persistently positive antiphospholipid antibodies (aPL) of any type (anticardiolipin [aCL] IgM/IgG or anti-β2 glycoprotein I [anti-β2GPI] IgM/IgG) or lupus anticoagulant (LA). APS may be primary or associate other rheumatic diseases, mostly SLE [3].

Anti-β2-glycoprotein I antibodies are formed against a phospholipid-binding plasma protein known as β2-glycoprotein I or apolipoprotein H [4]. The relation between anti-β2GPI and APS manifestations is of greater strength compared to that of aCL [5] and testing for anti-β2GPI is indicated in patients with APS manifestations but with negative aCL and LA [6].

Although classification criteria of APS don’t include IgA isotype of anti-β2GPI antibodies, it was found that many SLE patients are positive for these antibodies [7,8]. Measuring IgA anti-β2GPI could be considered for assessing the risk of thrombosis, particularly the venous thrombosis, in SLE patients [6,9]. Moreover, Lakos and his colleagues detected a strong relation between elevated IgA...
anti-β2-GPI antibody level and venous thrombosis, heart valve disease, thrombocytopenia, epilepsy and livedo reticularis [10]. Mehrani and Petri documented that, in the contrary to the IgG or IgM isotypes of anti-β2GPI, IgA isotype positivity had significant association with pulmonary hypertension. Hence, they suggested that a revision of the classification criteria of APS should be made to include IgA isotype [11].

A large number of lupus and APS patients as well as patients experiencing pregnancy morbidity or thrombosis without fulfilling the classification criteria of APS have positive IgA aPL with or without other aPL isotypes [10-13]. This motivated many researchers to keep evaluating the significance of aPL IgA isotype. They suggested that aPL IgA could help classifying patients having manifestations highly suggestive of the syndrome but lacking the standardized aPL. However, their work revealed controversial results [7,8,10,14-20].

Our study aimed at identifying the sensitivity and specificity of anti-β2GPI of IgA isotype among Egyptian lupus patients with and without APS.

**Patients and Methods**

The study included 54 Egyptian lupus patients. Age of the patients ranged from 13-46 years. The age of the control persons ranged from 14-45 years. Four of the patients, all with APS, were males. Ten of the enrolled patients, five with APS, had juvenile-onset SLE while the others developed lupus during adulthood. Age of onset ranged from 12-42 years. The range of disease duration was 0.6-23.3 years. SLE was diagnosed based on satisfying the 1997 update of the 1982 American College of Rheumatology revised criteria for SLE [21,22]. Half of the patients had secondary antiphospholipid syndrome (SAPS) based on satisfying the Revised preliminary Sapporo classification criteria of APS [23]. Group 1 patients refers to lupus patients without APS while group 2 patients refers to those with APS. The patients were recruited from the Rheumatology and Rehabilitation Department, Cairo University Hospital.

Risk factors of thrombosis as history of smoking, dyslipidemia, uncontrolled systemic hypertension, diabetes mellitus, nephrotic syndrome, use of estrogen containing contraceptive medications or hormone replacement treatment and other known thrombophilic disorders were excluded.

Twenty seven healthy control persons of matched age and sex were included. Local ethical committee approval was obtained.

Full history taking, clinical examination as well as routine laboratory investigations were done. Other investigations were performed when indicated.

**Patients and controls were evaluated for aPL positivity:**

- **Measurement of lupus anticoagulant level:** Screening was performed using the dilute Russell viper venom time (dRVVT). A confirmatory step followed screening. The screen ratio which is the ratio of screen clotting time of the tested plasma to that of the reference pool was calculated. The confirm ratio that is the ratio of confirm clotting time of the tested plasma to that of the reference pool was calculated as well. Lupus anticoagulant prolongation was confirmed if the normalized ratio, the ratio of the screen ratio to confirm ratio, was equal to or greater than 1.2. Kits was purchased from Diagnostica Stago, 9, rue des Frères Chausson 92600 ASNIERES, France and analyzer of the STA® line.

- **Measurement of anticardiolipin antibodies IgA, G and M levels:** ELISA kits was purchased from Demeditec Diagnostics GmbH, Lise-Meitner-Straße 2, D-24145 kiel (Germany). Test was considered positive when Ig A/G level was greater than 10U/ml and IgM level was greater than 7U/ml.

- **Measurement of anti-β2glycoprotein I IgA, G and M levels:** ELISA kits was purchased from Demeditec Diagnostics GmbH, Lise-Meitner-Straße 2, D-24145 kiel (Germany). Result was considered positive when the level of antibodies was greater than 8U/ml.

**Assessment of SLE activity and damage:**

Disease activity and damage were measured using Systemic Lupus Erythematosus Disease activity Index (SLEDAI) [24] and the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SLICC/SDI) respectively [25]. Both scores were measured at the time of aPL testing.

**Statistical methods:**

Accuracy of testing of IgA isotype of anti-β2GPI was expressed as sensitivity and specificity. Sensitivity was calculated using the following equation:

\[
\text{Sensitivity} = \frac{\text{Number of true positives}}{\text{Number of true positives} + \text{Number of false negatives}}
\]
Specificity was calculated using the following equation:

\[ \text{Specificity} = \frac{\text{Number of true negatives}}{\text{Number of true negatives} + \text{Number of false positives}} \]

P-values less than 0.05 was considered statistically significant. p.

Results

Table (1) shows the demographic features and obstetric history of the two patient groups and controls.

Comparison between the two patient groups concerning the clinical manifestations as well as disease indices revealed the existence of statistically significant differences regarding APS nephropathy, venous thromboses, DVT, superficial thrombophlebitis, arterial thrombosis, digital gangrene / threatened digital ischemia and Lymphopenia where these features were more frequent in group 2 patients (p-value: 0.038, 0.002, 0.002, 0.038, 0.002, 0.038 and 0.043 respectively). Dry mouth, discoid rash and cutaneous vasculitis were more frequent in group 1 patients than group 2 patients (p-value: 0.038, 0.017 and 0.026 respectively). SLICC-SDI was of higher value in group 2 patients than group 1 patients (p-value: 0.028) while there was no significant difference concerning SLEDAI.

Comparison between disease indices of the studied patient groups is shown in Table (2).

Routine laboratory features of the patients groups are shown in Table (3) with the comparison between the two groups.

Immunological laboratory features of the two patient groups and control with the comparison between the two patient groups and the comparison between the patients and the controls are shown in Table (4).

Table (1): Demographic features and obstetric history of the two patient groups and controls.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Group 1 patients (SLE)</th>
<th>Group 2 patients (SLE with APS)</th>
<th>Control</th>
<th>Demographic features N (%)</th>
<th>p value</th>
<th>Feature</th>
<th>Obstetric History N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (SLE)</td>
<td>N=27</td>
<td>N=27</td>
<td>N=27</td>
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<td>Group 1 patients (SLE)</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Group 2 patients (SLE with APS)</td>
<td></td>
</tr>
<tr>
<td>Demographic features N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sex Males</td>
<td>0 (0)</td>
<td>4 (14.8)</td>
<td>3 (11.1)</td>
<td>0.1</td>
<td></td>
<td>History of pregnancy ever</td>
<td>18 (85.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pregnancy morbidity</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>27 (100)</td>
<td>23 (85.2)</td>
<td>24 (88.9)</td>
<td>1</td>
<td></td>
<td>Abortion</td>
<td>3 (16.7)</td>
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<tr>
<td>Disease onset:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juvenile onset</td>
<td>5 (18.5)</td>
<td>5 (18.5)</td>
<td>–</td>
<td>0.4</td>
<td></td>
<td>Stillbirth</td>
<td>3 (16.7)</td>
</tr>
<tr>
<td>Adult onset</td>
<td>22 (81.5)</td>
<td>22 (81.5)</td>
<td>0.89</td>
<td></td>
<td></td>
<td>IUGR</td>
<td>2 (11.1)</td>
</tr>
<tr>
<td>Age in years</td>
<td>27.06±5.83</td>
<td>29.09±7.5</td>
<td>27.6±7.3</td>
<td></td>
<td></td>
<td>Preterm labor</td>
<td>1 (5.6)</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>6.7±4.05</td>
<td>7.04±5.7</td>
<td>–</td>
<td></td>
<td></td>
<td>Pre-eclampsia</td>
<td>1 (5.6)</td>
</tr>
<tr>
<td>Demographic features Mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SLICC-SDI (Mean±SD)</td>
<td>0.78±1.12</td>
<td>8.22±8.9</td>
<td></td>
<td></td>
<td></td>
<td>SLEDAI (Mean±SD)</td>
<td></td>
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<tr>
<td>SLEDAI N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Inactive disease (≤3)</td>
<td>11 (40.7)</td>
<td>11 (40.7)</td>
<td></td>
<td></td>
<td></td>
<td>Severe flare (&gt;12)</td>
<td>5 (18.5)</td>
</tr>
<tr>
<td>Mild-moderate flare (4-12)</td>
<td>15 (55.6)</td>
<td>12 (44.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Severe flare (&gt;12)</td>
<td>0 (0)</td>
<td></td>
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</tr>
</tbody>
</table>

Table (2): Comparison between disease indices of the two patient groups.
Levels of antiphospholipid antibodies in patient groups and controls with the comparison between the two patient groups and the comparison between the patients and the controls are shown in Table (5).

The specificity and sensitivity of each of aPL were calculated. Regarding sensitivity of aPL among SLE patients with APS, anti-ß2GPI antibodies were the most sensitive (59.3%), followed by LA (40.7%) and aCL (22.2%). Among anti-ß2GPI isotypes, the most sensitive isotype was IgA (48.1%), followed by IgG and IgM (14.8% for each). Among aCL isotypes, the most sensitive isotype was IgG (14.8%) followed by IgM (11.1%) and lastly IgA (0%).

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### Table (3): Routine laboratory features of the two patients groups.

<table>
<thead>
<tr>
<th>Feature (Mean±SD)</th>
<th>Group 1 patients (SLE) (N=27)</th>
<th>Group 2 patients (SLE with APS) (N=27)</th>
<th>P value</th>
<th>Feature (Mean±SD)</th>
<th>Group 1 patients (SLE) (N=27)</th>
<th>Group 2 patients (SLE with APS) (N=27)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR (mm/1st hour)</td>
<td>50.33±35.97</td>
<td>33.52±24.6</td>
<td>0.084</td>
<td>Serum albumin (3.5-5.5gm/dL)</td>
<td>3.53±0.64</td>
<td>3.98±0.47</td>
<td>0.006</td>
</tr>
<tr>
<td>Hb (mg/dL)</td>
<td>11.31±1.77</td>
<td>12.08±1.95</td>
<td>0.21</td>
<td>Serum creatinine (0.4-1.2mg/dL)</td>
<td>0.69±0.21</td>
<td>0.83±0.45</td>
<td>0.993</td>
</tr>
<tr>
<td>TLC (cell/mm³)</td>
<td>6.97±3.31</td>
<td>6.63±1.69</td>
<td>0.959</td>
<td>Blood urea (10-50mg/dL)</td>
<td>27.3±10.71</td>
<td>28.11±13.32</td>
<td>0.515</td>
</tr>
<tr>
<td>PLT (cell/mm³)</td>
<td>252.22±77.53</td>
<td>285.15±72.95</td>
<td>0.186</td>
<td>Proteinuria during activity of nephritis (0.15gm/24 hours)</td>
<td>1.88±1.21</td>
<td>2.06±1.06</td>
<td>0.422</td>
</tr>
<tr>
<td>AST (0-41 U/L)</td>
<td>24.21±11.53</td>
<td>22.63±10.21</td>
<td>0.678</td>
<td>Current level of proteinuria (0.15gm/24 hours)</td>
<td>0.86±0.85</td>
<td>0.98±1.11</td>
<td>0.631</td>
</tr>
<tr>
<td>ALT (0-41 U/L)</td>
<td>21.39±9.72</td>
<td>27.11±16.59</td>
<td>0.216</td>
<td>C3 (80-160mg/dL)</td>
<td>99.67±69.81</td>
<td>110.26±78.03</td>
<td>0.728</td>
</tr>
</tbody>
</table>

| Group 1 (N/%)     | 27/100                        | 26/96.3                                | 23/85.2 | 5/18.5 | 9/33.3 | 5/18.5 | 6/22.2 | 0/0 | 14/51.9 | 4/14.8 | 4/14.8 | 12/44.4 |
| Group 2 (N/%)     | 27/100                        | 27/100                                 | 23/85.2 | 11/40.7 | 6/22.2 | 3/11.1 | 4/14.8 | 0/0 | 16/59.3 | 4/14.8 | 4/14.8 | 13/48.1 |
| p-value of the comparison between the two patient groups | = 0.3                         | = 1                                    | = 0.07  | = 0.36 | = 0.44 | = 0.48 | = 0.584 | = 1 | = 0.79  |
| Control (N/%)     | = =                           | =                                     | = 1/3.7 | = 3/11.1 | = 2/7.4 | = 0/0 | = 1/3.7 | = 7/25.9 | = 0/0 | = 1/3.7 | = 6/22.2 |
| p-value of the comparison between the patients and the controls | = =                           | =                                     | = 0.006 | = 0.16 | = 0.48 | = 0.026 | = 0.33 | = 0.02 | = 0.047 | = 0.26 | = 0.052 |
The most specific antibodies for APS that can discriminate between SLE patients without APS and those with APS were in the following order: LA (81.5%), aCL (62.96%) and lastly anti-β2GPI (48.1%). Among anti-β2GPI isotypes, the most specific isotype was IgM (85.2%), followed by IgG (85.2%) and lastly IgA (55.6%). Among aCL isotypes, the most specific isotype was IgA (100%) followed by IgM (81.5%) and lastly IgG (77.8%).

When specificity is calculated using test results of the controls, the most specific antibodies were in the following order: LA (96.3%), aCL (88.9%) and lastly anti-β2GPI (74.1%). Among anti-β2GPI isotypes, the most specific isotype was IgM (100%) and IgG (96.3%) and lastly IgA (77.8%). Among aCL isotypes, the most specific isotype was IgG (100%) followed by IgA (96.3%) and lastly IgM (92.3%).

**Discussion**

Antiphospholipid antibodies of IgA isotype were not included in APS classification criteria due to lacking specificity and providing no additional benefit to those given by IgM or IgG isotypes [8]. Being not fully standardized makes it difficult to compare studies performed by different laboratories [11,26-29]. Moreover, there is a significant difference among the studied performed on different ethnicities providing further difficulty to the comparison of the different studies [30-33]. Furthermore, most of the studies supporting the value of IgA aPL are of different designs. In addition, these antibodies are frequently associated with other aPL [34].

Our study aimed at identifying the sensitivity and specificity of anti-β2GPI of IgA isotype among Egyptian lupus patients with and without APS. The different isotypes of anti-β2GPI were tested in an equal number of apparently normal age and sex-matched control subjects.

When specificity is calculated using test results of the controls, the most specific anti-β2GPI isotype was IgM (100%), followed by IgG (96.3%) and lastly IgA (77.8%).

On the contrary to our results where 22.2% of normal persons had anti-β2GPI IgA, none of the control subjects had anti-β2GPI IgA in a study done by Lakos and his colleagues [10].

The most specific anti-β2-GPI isotype that can discriminate between SLE patients without APS and those with APS was IgM (85.2%), followed by IgG (85.2%) and lastly IgA (55.6%).

We detected no difference in the prevalence and level of anti-β2GPI IgA between lupus patients without and with APS. In disagreement with our results, Fanopoulos and his colleagues reported that IgA anti-β2GPI were more prevalent and had in a higher level in SLE patients with APS than in those without APS [18]. In a study of 70 patients with SLE, 3 women with primary APS and 30 with secondary APS, both the frequency and the level of aPL were significantly higher in patients with SLE with APS than those without; this significant difference was stronger for IgA than IgG and IgM [10].

On the contrary, it was reported that aPL of IgG isotype are associated with a higher risk of thrombosis than other isotypes, IgM and IgA [35-37]. Moreover, Samarkos and his colleagues found that the addition of IgA aPL to the diagnostic tests of APS, even, decreased the accuracy of the test [35].

Racial differences were considered to have a role in the frequency, isotype distribution and clinical value of aCL and anti-β2-GPI [31].

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**Table (5): Levels of antiphospholipid antibodies in patient groups and controls.**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Diluted RVVT (Prolonged if ≥1.2)</th>
<th>aCL IgM (normal: &lt;7U/mL)</th>
<th>aCL IgG (normal: &lt;10U/mL)</th>
<th>aCL IgA (normal: &lt;10U/mL)</th>
<th>Anti-β2GPI IgM (normal: &lt;8U/mL)</th>
<th>Anti-β2GPI IgG (normal: &lt;8U/mL)</th>
<th>Anti-β2GPI IgA (normal: &lt;8U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Mean±SD)</td>
<td>0.78±1.12</td>
<td>6.11±13.3</td>
<td>9.37±19.28</td>
<td>1.87±2.16</td>
<td>6.79±15.67</td>
<td>3.09±2.04</td>
<td>11.3±13.13</td>
</tr>
<tr>
<td>Control (Mean±SD)</td>
<td>0.136</td>
<td>0.853</td>
<td>0.715</td>
<td>0.427</td>
<td>0.841</td>
<td>0.301</td>
<td>0.597</td>
</tr>
<tr>
<td>p-value of the comparison between the two patient groups</td>
<td>0.001</td>
<td>0.163</td>
<td>0.021</td>
<td>0.009</td>
<td>0.056</td>
<td>0.073</td>
<td>0.048</td>
</tr>
</tbody>
</table>
Prospective studies are mandatory to confirm the clinical usefulness of IgA isotype of anti-ß2GPI in different races [5].

From our findings, we can conclude that testing for IgA anti ß2GPI in SLE patients with manifestations suggestive for APS, but having none of the standardized aPL could be helpful owing to its high sensitivity. However, more studies on a larger number of patients excluding those with inherited thrombophilias, although this may not be feasible in anticoagulated patients, are needed.

References


22- HOCHBERG M.C.: Updating the american college of rheumatology revised criteria for the classification of


