Serum levels of anti-β₂-glycoprotein-I antibodies and anti-cardiolipin antibodies in children with systemic lupus erythematous

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Background: There are few reports on the relationship of anti-β₂-glycoprotein-I antibodies and anticardiolipin antibodies in children with systemic lupus erythematous (SLE). This study was undertaken to compare the serum levels of anti-β₂-glycoprotein I (β₂-GPI) antibodies with those of anticardiolipin (aCL) antibodies in SLE patients with secondary antiphospholipid syndrome (SAPS) and without SAPS (WSAPS).

Methods: Forty-two SLE patients with SAPS and 68 without SAPS were studied. Serum aCL antibodies and anti-β₂-glycoprotein I antibodies were measured by ELISA.

Results: The serum level of anti-β₂-GPI antibodies in 57.1% (24/42) of the patients in the SAPS-SLE group was higher than that in the control group, whereas it was only 1.5% (1/68) in the WSAPS-SLE group (P < 0.01). The serum level of aCL antibodies was higher in 6.68% (28/42) of the patients in the SAPS-SLE group and in 42% (29/68) in the WSAPS-SLE group (P < 0.01).

Conclusions: Anti-β₂-glycoprotein I (β₂-GPI) antibodies are not only strongly associated with SAPS in children with SLE but also highly specific in predicting SAPS-SLE in comparison with aCL antibodies.


Key words: anti-β₂-glycoprotein I; platelet; anticardiolipin antibodies; thrombopeny; hemolysis; children; SLE

Introduction

Antiphospholipid syndrome (APS) is often found in patients with thrombus, platelet counts dropping, hemolysis, spontaneous abortion during metaphase, late pregnancy or increased level of antiphospholipid antibodies (aCL). The syndrome is divided into primary and secondary ones. The patients with systemic lupus erythematous (SLE) often present symptoms with secondary APS. Further research into SLE has indicated that a kind of negatively charged anticardiolipin antibodies and lupus anticoagulants in the sera of patients with secondary antiphospholipid syndrome (SAPS)-SLE (LAC) are associated with lipid proteins. Additionally, another kind of blood plasma lipid protein, namely β₂-glycoprotein I (β₂-GPI), can also react with blood plasma lipid protein. It is indicated that anti-β₂-GPI antibodies are closely related to a variety of clinical symptoms and progression of the disease. In the present study, we investigated the isotypes of anti-β₂-GPI antibodies in 110 children with SLE by the method of ELISA, and then analyzed a possible role of the isotypes of anti-β₂-GPI antibodies in children with SAPS-SLE.

Methods

Subjects

A total of 110 children with SLE were recruited at our hospital from January 2000 to September 2003, including 14 males and 96 females, aged from 5 to 17 years. They were all diagnosed according to the diagnostic criteria for SLE and APS established by the American Rheumatism Academy (ARA) in 1998. Based on their clinical symptoms and laboratory findings, the children were grouped into two groups. One group consisted of 42 children with platelet count dropping, thrombosis, hemolysis, anti-aCL antibodies and anti-LAC antibodies positive or negative, and another group comprised 68 children with SLE but SAPS-SLE
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and the above manifestations.\(^\text{[1]}\)

**Control group**

Normal control group was composed of 37 children aged from 3 to 15 years who were scheduled to perform operation. They had normal liver function and normal blood coagulation. Rheumatoid arthritis control group consisted of 28 children aged from 4 to 12 years with all kinds of rheumatoid arthritis enrolled from inpatient and outpatient departments and follow-up patients.

**Methods**

**Detection of anti-β2-GPI antibodies**

Purified β2-GPI was diluted into 10 mg/L by PBS buffer. Seventy-five μL of the solution was placed onto every well in a 96-well polystyrene plate, which were incubated subsequently with 2% BSA. Blood serum at 1:100 dilution, standard substances 2, 20, 200 RU/ml (RU/ml indicates relative unit per ml), and positive and negative controls were all added into the reaction plate. After incubation for an hour at room temperature, reaction liquid was covered with anti-human IgG, IgM, IgA signed by enzyme, substrate and terminating liquid. Optical densitometer was read at the wavelength of 450 nm. The concentration of standard substance was made for x-axis and absorbency for y-axis. Cut-off values of anti-β2-GPI IgG, IgM, IgA were all 20 RU/ml.

**Test of aCL antibodies**

All samples were determined for aCL antibodies IgG, IgM, IgA by ELISA. Anti-aCL antibodies IgG, IgM, IgA were typed in all the subjects. Standard curves were made respectively according to the concentration of standard substances 2, 12, 120 U/ml for x-axis and absorbency for y-axis. The absorbency of detected sample was read from the standard curve. aCL antibodies IgG, IgM, IgA were grouped into 4 groups according to their values: negative for value less than 12, low positive for 12-19, moderate positive for 20-79, and strongly positive for higher than or equal to 80.

**Test of LAC**

This test was performed according to the reported method.\(^\text{[2]}\)

**Dynamic observation**

Six hospitalized SAPS-SLE children were randomly chosen for observation of dynamic changes of aCL antibodies and anti-β2-GPI antibodies from 0 to 10 days.

**Statistical analysis**

Data in the present study were analyzed by student’s t test and SPSS 10.0 software. A P value of less than 0.05 was considered statistically significant.

**Results**

**Comparison of antibodies of aCL and anti-β2-GPI among the groups**

Twenty-five (22.7%) of the 110 children with SLE and 24 (57.1%) of the 42 children with SAPS-SLE showed an increase of anti-β2-GPI antibodies. Fifty-seven (51.8%) of the 110 children and 28 (66.7%) of the 42 children with SAPS-SLE showed an increase of aCL antibodies.

Twenty-four (57.1%) of the 42 patients with anti-β2-GPI antibodies positive showed SAPS-SLE with aCL antibodies positive, while none of the patients with aCL antibodies negative SAPS-SLE and WSAPS-SLE showed anti-β2-GPI antibodies positive; but β2-GPI antibodies were significantly related to aCL antibodies ($P < 0.01$).

In the WSAPSE-SLE group, one (1.5%) of the 68 patients showed an increased level of antibodies of β2-GPI, and 29 (42.7%) an increased level of aCL antibodies. The specificity of anti-β2-GPI antibodies increased to 98.5%, whereas the specificity of aCL antibodies was only 57.3% ($P < 0.01$).

**Table 1. Comparison of SAPS, WSAPS and control groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>$n$</th>
<th>Positive ($n$)</th>
<th>Rate of positivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>42</td>
<td>23</td>
<td>66.7(^*)</td>
</tr>
<tr>
<td>SAPS</td>
<td>68</td>
<td>39</td>
<td>42.7(^*)</td>
</tr>
<tr>
<td>WSAPS</td>
<td>37</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rheumatoid arthritis control</td>
<td>28</td>
<td>9</td>
<td>32.1(^*)</td>
</tr>
</tbody>
</table>

Comparison of aCL antibodies: \(^*\) compared with normal control, $P < 0.01$; \(\Delta\) compared with rheumatoid arthritis control group, $P < 0.01$.

**Table 2. Dynamic comparison of isotypes of anti-aCL and anti-β2-GPI antibodies in 6 SAPS-SLE patients**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Day 0</th>
<th>Day 10</th>
<th>Anti-β2-GPI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG</td>
<td>IgM</td>
<td>IgA</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- - negative; +, low positive; ++, moderate positive; ++++, strongly positive.

**Isotypes of anti-aCL and anti-β2-GPI antibodies**

Isotypes of anti-aCL and anti-β2-GPI antibodies were determined in 6 children on day 0 and 10 (Table 2). All the 6 patients showed an increased level of IgM of anti-aCL and anti-β2-GPI antibodies on day 0, whereas
the level of IgG was negative or low positive. On day 10, the level of IgG increased while the level of IgM decreased. The level of IgA was not changed between the 10 days.

**Discussion**

We determined the isotypes of anti-β₂ GPI antibodies of SLE patients and SLE patients with SAPS. The level of anti-β₂ GPI antibodies increased in the SAPS-SLE patients with aCL antibodies positive, and the level of β₂ GPI antibodies was related to aCL antibodies. As a seromarker, however, anti-β₂ GPI antibodies were more specific than aCL antibodies to judge whether the patients have APS. Recent studies have shown that β₂-GPI is essential to APS. A special bound site of phospholipid exists in the position cystines 281-288 of β₂-GPI peptide chain, which can cognate with aCL antibodies. Cardiolipin monoclonal antibodies purified from a SLE mice model and serum of patients who are aCL antibodies positive can be bound with β₂-GPI in the absence of cardiolipin, confirming that β₂-GPI contributes to the development of APS.

aCL antibodies positive are markers of SLE diagnosis. In the present study, 25 patients also showed elevated levels of β₂-GPI antibodies and aCL antibodies. About 96% of the patients had SAPS-SLE. In the group of aCL antibodies negative (LAC positive and have or not SAPS), β₂-GPI antibodies were not detected. In the groups of SLE and WSAPS (with or without antibodies), Only 1 patient had an increased level of β₂-GPI antibodies. The results showed that β₂-GPI antibodies are not only closely related to aCL antibodies but also to the development of APS and other clinical symptoms such as thrombus, thrombocytopenia and hemolysis. In the group of WSAPS-SLE, 1.5% of them were β₂-GPI antibodies positive, but 42.8% were aCL antibodies positive. Thus, β₂-GPI antibodies are better than aCL antibodies in judging whether or not SLE patients have APS. The finding may be explained that polyclonal B cell is activated to produce some "non-specific anti-phospholipid antibodies", which are not related to SLE pathogenesis. In the control group of this study, 32.1% showed a slightly increased level of aCL antibodies (12-19 U/L). We speculate that the increased level of aCL antibodies in the group of WSAPS-SLE may be related to the damage of cell membrane.

We found that β₂-GPI could interact negatively with charged proteins and play an important role in APS with thrombosis, thrombocytopenia, hemolysis and spontaneous abortion during metaphase or later. Under normal conditions, β₂-GPI can suppress ADP-dependent platelet aggregation, triggering of blood coagulation factor XII, and blood coagulation. The production of anti-β₂-GPI antibodies either for extrinsic or intrinsic factors may change the structure of β₂-GPI or expose the binding site.

In the present study, we focused on the relationship between β₂-GPI antibodies and the symptoms of thrombosis, thrombocytopenia, and hemolysis. Because the patients were all under 18 years old, we could not find the correlation of β₂-GPI antibodies and abortion.

Moreover, 14.3% of the SAPS-SLE patients with aCL antibodies positive were not detected with an increased level of anti-β₂ GPI antibodies. We suggested that aCL antibodies tested by ELISA are useful to detect APS. Despite non-specific deficiency, the method is very sensitive. aCL antibodies test should be taken as a routine method for the diagnosis of APS, and β₂-GPI antibodies for determination of risk rate of patients with APS.

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**References**

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