Clinical Significance of Anti-streptococcal Esterase (ASE) Determination in Rheumatic Fever and Other Streptococcal Diseases

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Streptococcal esterase is known as one of the extracellular products of Group A streptococci. In this paper, an antibody of streptococcal esterase titers in patients with rheumatic fever, acute glomerulonephritis and other streptococcal infections were determined. In cases with acute rheumatic fever and acute glomerulonephritis, ASE titers showed almost the same changes as ASO, ASK and ADN-B. The characteristics of the ASE determination, however, is that small changes in the titers could be detected because the final definition of this titer was determined by photometer.

GROUP A streptococci produce many kinds of extracellular antigens including streptolysin O, streptokinase, hyaluronidase, deoxyribonuclease, esterase and others. The method for determination of human serum antibodies for streptococcal esterase (ASE) was first reported by Stock et al. in 1969.

In 1977, Hayano and Tanaka devised a new method to determine the antibody for this antigen by repetitive counterelectrophoresis. A few papers have been published concerning ASE titers using this method.

The purpose of this paper is to determine anti-esterase titers and to compare them with other streptococcal antibodies in cases of rheumatic fever and other streptococcal infections.

MATERIAL AND METHODS

The serum samples used in this study were obtained from patients with rheumatic fever, acute glomerulonephritis, scarlet fever, vascular purpura, chance hematuria and healthy controls. The serum samples from both “acute” rheumatic fever and glomerulonephritis patients were taken within 3 months of the onset of the diseases. The anti-streptococcal esterase (ASE) titers were determined using an enzyme antigen-antibody reaction (Fig. 1). To separate the complex of streptococcal esterase and the antibody of streptococcal esterase from the superfluous enzyme and serum components, cell wall debris of staphylococcus aureus, strain Cowan I, was used as absorbent. To develop the enzymatic activity of esterase bound with the anti-esterase and staphylococcus cell wall, S-acetylthiophenol was used as a substrate and 2-nitrobenzoic acid as a coloring reagent. These determination kits were available commercially from Dainippon Pharmacy Co. in Japan. The determination of anti-streptococcal polysaccharide (ASP) was made using an ASP kit (Hoechst Japan).

RESULTS

The results of the ASE titer in 345 control children, aged 6 to 15, are shown in Table I.

In general, the normal limit of streptococcal antibodies is considered to be in the upper 15 to 20% of the examined group. Consequently, the upper limit of ASE in normal children in this study was 400 units.

Key Words:
ASE (anti-streptococcal esterase)
ASO (anti-streptolysin-O)
ADN-B (anti-deoxyribonuclease B)

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TABLE I DISTRIBUTION OF THE ASE TITER IN SCHOOL CHILDREN
(6-15 years of age, 354 children)

<table>
<thead>
<tr>
<th>ASE titer</th>
<th>50 or under</th>
<th>51</th>
<th>101</th>
<th>151</th>
<th>201</th>
<th>251</th>
<th>301</th>
<th>351</th>
<th>401</th>
<th>451</th>
<th>501</th>
<th>601 or over</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of children</td>
<td>64</td>
<td>33</td>
<td>41</td>
<td>33</td>
<td>37</td>
<td>27</td>
<td>12</td>
<td>23</td>
<td>30</td>
<td>21</td>
<td>27</td>
<td>6</td>
</tr>
<tr>
<td>Percentage</td>
<td>18.1</td>
<td>9.3</td>
<td>11.6</td>
<td>9.3</td>
<td>10.5</td>
<td>7.6</td>
<td>3.4</td>
<td>6.5</td>
<td>8.5</td>
<td>5.9</td>
<td>7.6</td>
<td>1.7</td>
</tr>
</tbody>
</table>

23.7% 15.2% 9.3%

DETERMINATION OF ANTI-STREPTOCOCCAL ESTERASE

- Serum
  - Streptococcal esterase
  - Staphylococcal protein A
  - Incubate for 30 min. (37°C)
  - Add saline water
  - Centrifuge for 10 min. (1500G)
  - Remove upper layer
  - Add saline water
  - Centrifuge for 10 min. (1500G)
  - Remove upper layer
  - Add coloring agent (5,5'-dithiobis-(2-nitrobenzoic acid)
  - Add substrate (S-acetyl thiophenol)
  - Incubate for 30 min. (37°C)
  - Add stopping agent (succinate-sod. borate buffer)
  - Centrifuge for 10 min. (1500G)
  - Determine by photometer (412nm)

Fig.1. Determination of anti-streptococcal esterase.

The mean titer of the 6 to 8 year old age group was 210u.. In the 9 to 11 year old age group, it was 200u., and in the 12 to 15 year old age group, it was 267u.. The results of upper normal limits of other streptococcal anti-bodies in the control group were as follows: ASO: 160u., ADN-B: 480u., ASK: 640u. and ASP: 32u..

Of 237 school children who had micro-hematuria determined by repeated urinalysis (chance hematuria), 28% had ASE titers higher than 401u. The ASO titers were high in 23% of them (Table II). The ASK titers were high in 28% and the ADN-B titers were high in 21% and the ASP titers were high in 24% of these children.
TABLE II  RESULTS OF STREPTOCOCCAL ANTIBODIES IN PATIENTS WITH STREPTOCOCCAL DISEASE (%)

<table>
<thead>
<tr>
<th>Disease</th>
<th>ASE  &gt; 401</th>
<th>ASO  &gt; 240</th>
<th>ASK  &gt; 1280</th>
<th>ADN-B  &gt; 640</th>
<th>ASP  &gt; 64</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.7</td>
<td>21.0</td>
<td>22.0</td>
<td>22.3</td>
<td>15.0</td>
</tr>
<tr>
<td>Chance hematuria</td>
<td>28</td>
<td>23</td>
<td>28</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>Rheumatic fever</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>within 3 months</td>
<td>63</td>
<td>75</td>
<td>75</td>
<td>63</td>
<td>63</td>
</tr>
<tr>
<td>after 4 months</td>
<td>27</td>
<td>33</td>
<td>27</td>
<td>27</td>
<td>13</td>
</tr>
<tr>
<td>Nephritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>within 3 months</td>
<td>69</td>
<td>69</td>
<td>54</td>
<td>62</td>
<td>85</td>
</tr>
<tr>
<td>after 4 months</td>
<td>60</td>
<td>30</td>
<td>13</td>
<td>47</td>
<td>67</td>
</tr>
<tr>
<td>Vascular purpura</td>
<td>20</td>
<td>13</td>
<td>17</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Scarlet fever</td>
<td>33</td>
<td>33</td>
<td>17</td>
<td>0</td>
<td>50</td>
</tr>
</tbody>
</table>

TABLE III  PERCENTAGE OF PATIENTS WHOSE SERA WAS "POSITIVE"

<table>
<thead>
<tr>
<th>ASO, ASK, ADN-B</th>
<th>ASO, ASE, AND-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Rheumatic fever</td>
<td>94</td>
</tr>
<tr>
<td>Acute Nephritis</td>
<td>82</td>
</tr>
<tr>
<td>Vascular purpura</td>
<td>69</td>
</tr>
</tbody>
</table>

1  = at least one of the three were elevated  
2  = more than two of the three were elevated  
3  = all three were elevated

There was no significant difference in titration between the control group and the children with hematuria. Two of the chance hematuria children showed high streptococcal antibodies in all five (ASE, ASO, ASK, ADN-B and ASP). Twelve children showed high titers in four of the five antibodies, 25 children showed high titers in two of the five streptococcal antibodies. Consequently, 39 children (16%) showed high titers in three or more of the five antibodies and 85 children (36%) showed high titers in two or more of the five antibodies. In these cases, streptococcal infection may play an important role in the etiology of this condition.

The cases with rheumatic fever were divided into two groups, i.e. active stage and inactive stage. In patients with active rheumatic fever, there were high ASE titers in 6 of the 8 cases (7 samples from 11). Percentage of patients showing high titers: ASO-75%; ASK-75%; ADN-B-63% and ASP-63%.

In patients with inactive rheumatic fever who were receiving penicillin for prophylaxis, ASE titers were in the elevated range in 27% of the cases.

In patients with acute glomerulonephritis, 69% showed high ASE and ASO titers and 54% showed high ASK titers, 62% showed high ADN-B titers and 85% showed high ASP titers.

In patients with vascular purpura, 20% showed high levels in ASE, 13% showed high levels in the ASO and ASK titers, 47% showed high ADN-B titers and 67% showed high ASP titers.

In patients with scarlet fever, 33% showed high levels in the ASE and ASO titers.

In rheumatic fever, within 3 months of the onset of the disease, 94% of the patients showed a high level and in acute nephritis, 82% of the patients showed a high level in at least one of the three antibodies, that is ASO, ASK and ADN-B. In the determination of ASO, ADN-B and ASE, 85% of the cases showed a high level in acute

*Japanese Circulation Journal Vol. 48, December 1984*
rheumatic fever and 94% of the cases showed a high level in acute nephritis (Table III).

DISCUSSION

Group A streptococci produce various kinds of extracellular antigens including streptolysin O, streptokinase, hyaluronidase, deoxyribonucleases and esterase.

Antibody determination for these antigens is clinically designated as ASO, ASK, AHD, ADN-B and ASE, and as an antibody for cellular antigen, the antistreptococcal polysaccharide level (ASP) has been recently determined. The antibody titers were influenced by the strain of streptococci, the age of the patients, early and proper treatment and sensitivity to the antigen. When patients were infected by the strains which produce a small amount of streptolysin O, the antibody titers of ASO in these patients may not show a high level.

We can see the same kind of results when patients are given early treatment for scarlet fever; antibody titers for streptococcal antigens may show a rather lower level.

Because all these antibodies are serological reactions, one should detect and examine these antibodies throughout the course of the disease; for example, in the acute phase, a few in the convalescent stage ect.

If there is more than a two dilution difference in the titers, one can establish recent streptococcal infection. Therefore, to make the correct diagnosis for streptococcal infection serologically, we have to wait a few weeks for the elevation of the titration. If one is to make a definite diagnosis after the patient’s first visit, one has to observe an extremely high titer. But when the titer is within the upper limit of the normal or near-normal range, serological diagnosis is difficult. In such a situation, it is difficult to make a definite diagnosis by a serological test, when we determine only one antibody, eg. ASO. I recommend examining three antibodies at the same time, especially on the first visit of the patients; when two of the three titers show a high level, we can make a definite diagnosis of streptococcal infection.

In this sense, the method of determination for ASE titers is made by a photometer, which is capable of registering even a small change in the titer.

In this way, 82 to 94% of the patients were found to have high ASE, ASO and ADN-B or ASO, ASK and ADN-B in acute rheumatic fever and acute glomerulonephritis.

SUMMARY

Antibody titers for streptococcal esterase were determined and the following results were obtained:

1. The mean titers of anti-streptococcal esterase in control children between 6 and 15 years of age were 230 units.
2. The upper normal limits of this control group were 400 units.
3. In cases with acute rheumatic fever and glomerulonephritis, ASE titers showed almost the same changes as ASO, ASK and ADN-B.
4. There was no disease specificity concerning the titers of ASE.
5. The correlation between titers among 126 streptococcal infected patients were as follows; ASE-ASO: $\gamma = 0.635$, ASE-ASK: $\gamma = 0.577$, and ASE-ADN-B: $\gamma = 0.404$.
6. ASE titration was determined by a photometer, so that small changes in the titer could be observed.

REFERENCES