Antithrombin III Activity in Cerebrovascular Accidents


Abstract

Cerebrovascular accidents are commonly due to occlusive or haemorrhagic lesions. The present prospective study was planned to find out role of antithrombin in possible etiopathological process, which might predispose an individual for stroke.

Method: Biological activity of antithrombin III was done by the method as described by Innerfield et al (1976). Immunological estimation of antithrombin III was done by single radial immunodiffusion by the technique of Mancini et al modified by Fahey and McKelvey.

Results: The biological and immunological activity of antithrombin III was measured in 98 patient of occlusive and 56 patients of haemorrhagic strokes.

Significant depression in biological as well as immunological activity (p<0.001) was observed in occlusive stroke. In haemorrhagic stroke both, biological and immunological activity was increased.

In follow up study, there was progressive normalization of both, biological as well as of immunological activity in both group.

Conclusion: Decrease of antithrombin III in occlusive and increase in haemorrhagic stroke indicates that these changes have at least an additive role in the pathogenesis of stroke.

INTRODUCTION

Antithrombin III (AT III) a potent physiological anticoagulant present in the blood; acts by inhibiting the enzymatically active form of vitamin-K dependent clotting factors. Egeberg1 first reported a Norwegian family with congenital deficiency of AT III, presenting with repeated episodes of thromboembolism. In 1964, Ettinger et al2 while studying the hypercoagulability in patient with non-haemorrhagic stroke, reported decreased AT III; the most constant test abnormality. This aroused new interest in the field of blood coagulation and various associated diseases. The speed of clotting on one hand and the speed of clot lysis on the other hand are critical and that considerable change in this delicate ‘haemostatic balance’ produce clinical symptoms, either haemorrhage or thromboembolism. Cerebrovascular accident (CVA), one of the common neurological disorder have been studied with a view to elucidate the role of AT III in initiation of sudden onset occlusion or haemorrhage.

MATERIAL AND METHODS

This study included 98 patients with occlusive and 56 patients with haemorrhagic strokes. The diagnosis was based on detailed history, clinical examination and was confirmed by CT scan of brain. All the patients were studied within first week of onset of stroke but follow-up, studies during second week (T2 stage) and after one month (T3 stage) were possible only in 31 patients with occlusive stroke and 12 patients with haemorrhagic stroke. Sixty five age and sex matched healthy subjects served as controls.

Biological activity of AT III was estimated by the method of Innerfield et al.3 Briefly after standardization of thrombin (Park-Devis) biological activity of AT-time was recorded in seconds. AT III activity was calculated with the help of standard graph obtained simultaneously from normal pooled sera representing 100%, 75%, 50% and 25% AT III activity.

Immunological estimation of AT III was done by single radial immunodiffusion technique as described by Fahey and McKelvey.4 Briefly, after preparing agar-antithrombin antiserum mixture was poured directly over the glass slides and allowed to solidify, there after kept in humid chamber for overnight. The wells of 2 mm at a distance 14 mm apart were cut. To these wells 2 microliter patient’s sera was added and incubated for 18 hrs at room temperature. Under oblique illumination, diameter of rings was measured and readings
were taken on measuring scale (Hoechst) AT III activity was calculated with the help of standard graph obtained from normal pooled sera representing 100%, 75%, 50% and 25% AT III activity.

Statistical analysis was done by applying Student-t test.

**RESULTS**

Out of 98 patients in occlusive group 67 were male and 31 were female, whereas out of 56 haemorrhagic group 38 were male and 18 were female. In occlusive stroke, 31 patients were below and 67 were above 50 years of age, whereas in haemorrhagic stroke 14 were below 50 years while 42 were above 50 years of age. The biological and immunological activity was measured in 104 controls.

There was significant depression (p<0.001) in biological AT III activity in our patients with occlusive stroke (mean 84.3 ± 12.4%) as compared to health controls in different age group (Table 1 and 2). Similarly immunological AT-III activity was also found depressed (mean 76.4 ± 16.2%) in different age group (Table 1).

In contrast to occlusive stroke, our patients with haemorrhagic stroke, showed increase in both biological (mean 112.2 ± 15.2%) and immunological (mean 110.0 ± 18%) AT III activity as compared to healthy controls (Table 1).

There was no difference in biological activity amongst the control in different age group. However, immunological activity was slightly less in above the age of 70 years but it is not statistically significant (Table 2 and 3).

A positive correlation was observed between the biological and immunological activities of antithrombin III both in occlusive and haemorrhagic stroke.

There was no difference both in biological and immunological activity, in antithrombin III in males and females.

In the follow-up study, there was a progressive normalization of biological and immunological activity in 26 patients with occlusive stroke at second week and at one month from initial values. In haemorrhagic stroke, follow up study could be done only in 12 patients. Similar observation as in occlusive stroke was also observed in this group and at the end of one month in majority of the patients antithrombin activity both biological and immunological returned to near normal level (Table 4).

**DISCUSSION**

Antithrombin III, an alpha-2 globulin, is a most important physiological inhibitor of coagulation system. It inactivates thrombin in a time dependent reaction which can be greatly accelerated by heparin due to its heparin cofactor activity and beside inhibiting activated factor X it inhibits other serine proteases namely factor IXa, XIa, XIIa, kallikrein and complement components also. The measurement of the inhibitory effect of antithrombin III may be helpful in identification of disorder of blood coagulability occurring in wide spectrum of diseases. The role of antithrombin III in cerebrovascular accidents or ‘stroke’ which is one of the major killers in elderly population is poorly understood.

Various coagulation abnormalities occurring in the systemic circulation in patients with several thromboembolic
disorders, have been known. Ettinger in 1964 described hypercoagulability in nonhaemorrhagic stroke. Amongst several parameters of coagulation he studied, decreased antithrombin III was the most constant test abnormality. Since then various parameters of coagulation in stroke have been studied but without unifying results. In stroke, the role of antithrombin III is however, controversial. The variations in the antithrombin III protein concentration and biological activities have been found in stroke patients.  

Current methods for the measurement of antithrombin III depend on either its biological activities or immunological activity in both occlusive as well as haemorrhagic stroke. There is clear cut enhancement of stroke risk in women taking oral contraceptives and such women show a fall in antithrombin III from 10 days onward.

Current methods for the measurement of antithrombin III depend on either its biological activities or immunological protein concentration with the few rare exceptions of qualitatively deficient antithrombin III (Antithrombin III Budapest) and immunologically detectable thrombin-antithrombin III complex. A decreased antithrombin III protein concentration and activity has been suggested to be of great importance in the pathogenesis of thromboembolism. We found significant depression in the protein concentration with the few rare exceptions of antithrombin III from 10th day onward.

Table 3: Comparison of mean antithrombin immunological activity between occlusive stroke cases, haemorrhagic stroke cases and controls in their different age groups

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Occlusive (A)</th>
<th>Haemorrhagic (B)</th>
<th>Control (C)</th>
<th>A vs B</th>
<th>A vs C</th>
<th>B vs C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SD</td>
<td>n</td>
<td>Mean ± SD</td>
<td>T</td>
<td>df</td>
</tr>
<tr>
<td>&lt; 30</td>
<td>7</td>
<td>74.9 ± 14.4</td>
<td>4</td>
<td>110.0 ± 10.7</td>
<td>4.22</td>
<td>9</td>
</tr>
<tr>
<td>30-39</td>
<td>11</td>
<td>83.7 ± 18.4</td>
<td>2</td>
<td>104.0 ± 11.3</td>
<td>1.48</td>
<td>11</td>
</tr>
<tr>
<td>40-49</td>
<td>13</td>
<td>69.2 ± 18.5</td>
<td>8</td>
<td>120.6 ± 28.9</td>
<td>5.00</td>
<td>19</td>
</tr>
<tr>
<td>50-59</td>
<td>22</td>
<td>74.6 ± 15.3</td>
<td>19</td>
<td>112.0 ± 18.3</td>
<td>7.11</td>
<td>39</td>
</tr>
<tr>
<td>60-69</td>
<td>27</td>
<td>76.1 ± 15.6</td>
<td>17</td>
<td>105.6 ± 15.4</td>
<td>6.16</td>
<td>42</td>
</tr>
<tr>
<td>70 and above</td>
<td>18</td>
<td>80.1 ± 16.6</td>
<td>6</td>
<td>104.3 ± 8.1</td>
<td>3.40</td>
<td>22</td>
</tr>
<tr>
<td>Total</td>
<td>98</td>
<td>76.4 ± 16.2</td>
<td>56</td>
<td>110.0 ± 18.1</td>
<td>11.51</td>
<td>112</td>
</tr>
</tbody>
</table>

Table 4: Comparison of mean antithrombin biological and immunological activities of occlusive stroke cases after a followup of 7-10 days and 25-30 days with corresponding controls

<table>
<thead>
<tr>
<th>Activity</th>
<th>Type of stroke</th>
<th>Initia reading</th>
<th>After 7-10 days</th>
<th>After 25-30 days</th>
<th>Control (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological</td>
<td>Occlusive</td>
<td>84.3 ± 12.4</td>
<td>26 91.0 ± 12.0</td>
<td>26 99.0 ± 10.0</td>
<td>104 102.7 ± 9.9</td>
</tr>
<tr>
<td>Haemorrhagic</td>
<td>112.2 ± 15.2</td>
<td>12 117.9 ± 9.8</td>
<td>12 103.6 ± 13.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunological</td>
<td>Occlusive</td>
<td>76.4 ± 16.2</td>
<td>26 75.0 ± 10.0</td>
<td>26 89.0 ± 8.5</td>
<td>104 97.7 ± 8.3</td>
</tr>
<tr>
<td>Haemorrhagic</td>
<td>110.0 ± 18.1</td>
<td>12 112.2 ± 9.9</td>
<td>12 106.8 ± 9.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Presumably, little change occurs in $\alpha_1$ antitrypsin and $\alpha_2$ macroglobulin in stroke. Whether this change in AT III has casual relationship for the genesis of stroke is not certain. The change in opposite direction namely decrease of AT III in occlusive stroke and increase in haemorrhagic stroke could mean that these changes have at least an additive role in the pathogenesis of stroke. Normalization of AT III activity in follow-up period suggests that changes in AT III activity are of short duration. If the alteration started in prestroke period, it would be certainly of great significance in the precipitation of stroke, but this temporal factor is difficult to define.

Acknowledgement

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REFERENCES


