Prevalence of Anticardiolipin Antibodies In Various Thrombotic Conditions : A Hospital-Based Study

S Chandrashekhara, R Kirthi, Joyce Varghese

Abstract

Thrombosis is one of the leading cause of death, globally. Several conditions enhance the chances of thrombosis. Atheromatous vessels, polycythemia, lipid profile changes, oral contraceptives and prolonged bed rest are some conditions, which have been attributed to cause thrombosis. In addition to the above causes, there are a few other conditions wherein the patient has tendency to thrombose in normal vessels without any traditional risk factor of thrombosis. Usually this occurs in the younger age group and the condition is called thrombophilia. The presence of Anticardiolipin antibodies (aCL) is one of the most common acquired protein defects causing thrombosis. In addition, aCL has also been demonstrated to play an important role in other diseases like recurrent abortions and connective tissue diseases (CTD). The clinical syndrome where the patient has thrombosis or recurrent pregnancy loss with the presence of aCL or lupus anticoagulant (LA) is termed as anti-phospholipid syndrome (aPL). There are pre-defined criteria for making the diagnosis of aPL syndrome.

In India, a few studies have been conducted regarding the prevalence and the incidence of aCL. These studies have attempted to address the role of aCL in specific clinical conditions like young stroke, cortical venous thrombosis and systemic lupus erythematosus (SLE). The need to diagnose the cause for thrombosis has been highlighted by the recent reviews. In a setting, incidences of the various thrombotic conditions are not available.

In our centre, we screened 302 patients for aCL. These patients had thrombosis at different sites without any additional risk factor for thrombosis and it revealed a few interesting facts, which are presented.

INTRODUCTION

Thrombosis is one of the leading causes of death, globally. Several conditions enhance the chances of thrombosis. Atheromatous vessels, polycythemia, lipid profile changes, oral contraceptives and prolonged bed rest are some conditions, which have been attributed to cause thrombosis. In addition to the above causes, there are a few other conditions wherein the patient has tendency to thrombose in normal vessels without any traditional risk factor of thrombosis. Usually this occurs in the younger age group and the condition is called thrombophilia. The presence of Anticardiolipin antibodies (aCL) is one of the most common acquired protein defects causing thrombosis. In addition, aCL has also been demonstrated to play an important role in other diseases like recurrent abortions and connective tissue diseases (CTD). The clinical syndrome where the patient has thrombosis or recurrent pregnancy loss with the presence of aCL or lupus anticoagulant (LA) is termed as anti-phospholipid syndrome (aPL).

MATERIAL AND METHODS

Three hundred and two patients referred to the Dept. of Clinical Immunology for evaluation of thrombosis from August 1997 to December 2001 were retrospectively included in our study. All patients had de novo (in situ) thrombosis. Detailed history to exclude reversible precipitating causes like smoking, dyslipidaemias, hypertension, prolonged bed rest, diabetes mellitus, prolonged use of oral contraceptives, atherosclerosis,
cardiovascular or congenital heart disease, polycythemia and preceding infections were noted. Hence, the study predominantly included only those patients who needed further evaluation for the cause of thrombosis.

Detailed epidemiological and clinical data were obtained from hospital records and clinical examinations. The sera were analysed for aCL by enzyme linked immunosorbent assay (ELISA) using commercially available kits (Orgentek). The tests were conducted as per the instructions of the manufacturers. To briefly describe the method, the sera was diluted at 1:100 dilution and added to pre-coated plates along with reference standards provided by the manufacturer. The plates were washed and anti-human HRP conjugate followed by substrate added and the subsequent color developed was measured using ELISA reader at 450 nm.

Protein C, protein S (by ELISA using Corgenix kit) and AT III levels (by calorimetric analysis using Organon Teknika) were done in 38 of these cases. The results were interpreted using the cut-off values as prescribed by the manufacturers. aCL IgG values greater than 15.0 GPL was taken as high titres. The normal ranges for protein C, protein S and AT III were 59% - 137%, 73% - 151% and 56% - 115% respectively. Suitable corrections for use of anticoagulants were made as specified by manufacturer.

**RESULTS**

A total of 302 (151 females and 151 males) patients were included in the study. One hundred and thirty four patients were below age of 40 years (juvenile-onset thrombosis) and the remaining was above 40 years. The mean age was 35.3 years. The predominant sites of thrombosis at the time of reference are shown in Table 1. The incidence of aCL IgG positivity is illustrated in Table 2. Out of the 302 patients only 38 were investigated for protein C, protein S and Antithrombin III deficiencies in addition to aCL and the results are in Table 3.

**DISCUSSION**

In this study, aCL was found to be present in high titers in several thrombotic settings. Sixty-five (20.77%) patients had high titers of aCL. The majority of patients (90) were screened for de novo deep vein thrombosis (DVT). The most common site of thrombosis was the deep veins of the lower limb. Saxena R et al while screening for aetiology of young DVT found 5.3% and 2.8% of patients with DVT to have aCL and LA respectively. A similar study from Western India, by Ghosh K et al reported the incidence of aCL and LA to be 9.9% and 8.3% respectively in patients with DVT in young. In our series we found the levels to be slightly higher and 25.56% of the cases had high titres of aCL IgG. In venous thrombosis, protein C, protein S and AT III deficiencies were detected in 7.54%, 16.03% and 4.72% respectively, in the present study. In another comparable study, protein C, protein S, AT III deficiencies and high levels of aCL were seen in 9.5%, 6.5%, 2.6% and 9.9% of the cases respectively. Among those with DVT, eight patients had recurrent pulmonary embolism. In this group, six (62.5%) patients had high aCL titres.

In patients with peripheral arterial thrombosis only 3 (18.75%) had high aCL IgG. Only four out of the 20 patients were tested for protein S levels and two were detected to have protein S deficiency. In a study by de Godoy JM et al analyzing peripheral arterial thrombosis, high titres of aCL was found more than 28.8% of the patients. In the present study, 16 patients had peripheral arterial thrombosis and the mesenteric artery was the most common site.

In present series, 14 patients had cortical venous thrombosis (CVT) and two (14.29%) patients demonstrated high aCL IgG titers. R Christopher et al (1999), while analyzing the role played by aCL in CVT, found 22.6% of patients to have significantly high titres, in comparison to 3.2% of normal. The observation in the present study concurs with their observations.

### Table 1: Demographic data

<table>
<thead>
<tr>
<th>Site of thrombosis</th>
<th>No. of patients</th>
<th>Male</th>
<th>Female</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral venous thrombosis</td>
<td></td>
<td>44</td>
<td>46</td>
<td>90 (29.8)</td>
</tr>
<tr>
<td>Peripheral arterial disease</td>
<td></td>
<td>9</td>
<td>7</td>
<td>16 (5.29)</td>
</tr>
<tr>
<td>Combined arterial and venous disease</td>
<td></td>
<td>9</td>
<td>10</td>
<td>19 (6.29)</td>
</tr>
<tr>
<td>Cortical vein</td>
<td></td>
<td>4</td>
<td>10</td>
<td>14 (4.63)</td>
</tr>
<tr>
<td>CNS territory (ischaemic stroke)</td>
<td></td>
<td>28</td>
<td>24</td>
<td>52 (17.21)</td>
</tr>
<tr>
<td>Coronary artery</td>
<td></td>
<td>43</td>
<td>15</td>
<td>58 (19.2)</td>
</tr>
<tr>
<td>Connective tissue disease</td>
<td></td>
<td>6</td>
<td>24</td>
<td>30 (9.93)</td>
</tr>
<tr>
<td>Site not known</td>
<td></td>
<td>7</td>
<td>16</td>
<td>23 (7.61)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>151</td>
<td>151</td>
<td>302</td>
</tr>
</tbody>
</table>

### Table 2: aCL positivity in the different Sites of thrombosis

<table>
<thead>
<tr>
<th>Site of Thrombosis</th>
<th>No. of positive (n)</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral venous disorder</td>
<td>23 (90)</td>
<td>25.56</td>
</tr>
<tr>
<td>Peripheral arterial disease</td>
<td>3 (16)</td>
<td>18.75</td>
</tr>
<tr>
<td>Combined*</td>
<td>2 (19)</td>
<td>10.53</td>
</tr>
<tr>
<td>Cortical vein</td>
<td>2 (14)</td>
<td>14.29</td>
</tr>
<tr>
<td>CNS territory (Ischaemic stroke)</td>
<td>13 (52)</td>
<td>25.0</td>
</tr>
<tr>
<td>Coronary artery</td>
<td>8 (58)</td>
<td>13.79</td>
</tr>
<tr>
<td>Connective tissue disease</td>
<td>8 (30)</td>
<td>26.67</td>
</tr>
<tr>
<td>Site not known</td>
<td>3 (23)</td>
<td>13.04</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>65 (302)</td>
<td>20.77</td>
</tr>
</tbody>
</table>

*Peripheral and venous site. + Number screened.

### Table 3: Incidence of blood coagulation protein deficiencies

<table>
<thead>
<tr>
<th>Low/positive*</th>
<th>% Positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCL IgG</td>
<td>7</td>
</tr>
<tr>
<td>Protein C</td>
<td>7</td>
</tr>
<tr>
<td>Protein S</td>
<td>18</td>
</tr>
<tr>
<td>AT III</td>
<td>11</td>
</tr>
</tbody>
</table>

*Positive for aCL IgG
In the present series, among the 52 patients with ischemic stroke high titres of aCL IgG was seen in 13 (25%) patients. Nagaraj D et al found a prevalence rate of 23% of aCL in young stroke compared to 3.2% of the normal control. In another comparable study by Stanley et al, high titres of aCL were detected in 34% of the patients. This variation could be due to racial and ethnic factors.

Among the 58 patients presenting with thrombosis in the coronary artery, only eight (13.79%) were detected to have high levels of aCL in the present study. In Western literature, aCL accounts for approximately 18% of premature coronary thrombosis.

Antiphospholipid antibodies are present in over 30% of the western SLE patients and complications are seen in a significant subset of these patients. In our setting, there were 18 cases of SLE presenting with thrombophilia. One-third of these cases had elevated titres of aCL IgG. Other studies by Shrivastava et al and Jone HW et al demonstrated respectively 51% and 16.5% of the patients to have high levels of aCL IgG. A study conducted by Cucurull et al has reported that the prevalence of at least one of three isotypes (IgG, IgM, and/or IgA) of aCL in the African-American patients with SLE is 33%. Petri found that the prevalence of aPL using a polyclonal aCL that can detect all three isotypes, including LA, in Afro-American patients were lower (25%) than in whites (40%). Weidmann et al found aCL IgA to be the most prevalent among their patients with SLE. They found aCL IgA in 44% of the patients, two-thirds of whom were white. 22% had aCL IgA isotype exclusively. The present study has a higher percentage of positive cases since only patients presenting with thrombotic complications or recurrent pregnancy loss were included.

We analyzed 38 patients for protein C, protein S and AT III deficiencies in addition to aCL. We found slightly higher incidence of the deficiencies due to referral bias. The data was too small to analyze. Other conditions like activated protein C resistance, homocysteinuria have been proved to be other associated conditions. European population data indicates an increased activated protein C resistance (APC-R) has raised the yield of diagnosed coagulation abnormalities up to 64% in thrombosis patients and upto 8% in the general population.

There is a need for systematic study with reference to incidence of thrombosis, its mortality and morbidity and etiopathogenesis in Indian population.

Recent understanding of thrombosis and thrombophilic disorders has lead to specific recommendation for thromboprophylaxis in preventing mortality and morbidity from this preventable disaster. Anticardiolipin antibodies are the most common acquired thrombophilic defect. Epidemiological data of our population is required for evaluating the strategy for further research of thrombosis in this condition.

Acknowledgement

We thank Ms. Jyothi G and Ms. Shobha A for the help rendered to us.

References

17. Cucurull E, Garhavi AE, Diri E, Mendez E, Kapoor D, Espinoza LR. IgA anticardiolipin and anti-(2-glycoprotein 1 are the most prevalent isotypes in African American patients with SLE. Am J Clin Pathol 2000;114:627-32.


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**Announcement**

**AWARD SESSIONS**

1. Dr. DP Basu Young Award in Cardiology
2. E Merck Award
3. Dr. JN Berry Memorial Award and
4. Dr. MJ Shah Memorial Award in Tropical Medicine

There will be four award sessions at the 2004 Annual Conference of API at Hyderabad. The rule and regulations of these awards are as under:

1. Papers that are accepted for presentation in the Award Session at the Annual Conference will be divided subject-wise into four groups:

   GROUP I CARDIOLOGY   DP BASU YOUNG AWARD
   GROUP II CHEST DISEASES   E MERCK AWARD
   GROUP III OTHER SPECIALITIES   JN BERRY MEMORIAL AWARD
   GROUP IV TROPICAL MEDICINE   MJ SHAH MEMORIAL AWARD

   The Award of Dr. JN Berry Memorial Award and E. Merck Award are given in alternate years in Group II and III papers. At the 2004 Annual Conference at Hyderabad, Dr. JN Berry Memorial Award will be for ‘Other Specialities’ and E Merck Award for ‘Chest Diseases’. Dr. DP Basu Young Ward will be for ‘Cardiology’ and Dr. M. J. Shah Memorial Award for ‘Tropical Medicine’.

2. The competitor must be the first author of the paper submitted for presentation at the API sessions of the Annual Conference. A testimonial must be submitted from the Head of the institution that the major work has been done by the competitor. Papers which are previously presented or published will not be considered. The competitor should also give a written pledge stating that the work has not been presented or published before. He should be a member of API.

3. Dr. JN Berry Memorial and DP Basu Young Awards are worth Rs. 1000/- each. E Merck Award Rs. 2000/- and Dr. MJ Shah Memorial Award is worth Rs. 2500/-.

4. The upper age list of the competitor is 40 years.

5. The decision will be taken by a panel of judges appointed by the Governing Body of API.

6. The candidate must apply for the award and full manuscript of the paper will have to be submitted. The paper will be presented in separate award session.

7. Eight copies of full manuscript will have to be submitted to Dr. (Maj. Gen.) S. Venkataraman, President - Elect and Chairman Scientific Committee, APICON 2004, Flat No.137, Air Force & Naval Officers Enclave, Plot No.11, Sector - 7, Papan Kalan, Dwarka, New Delhi - 110 045 of API by 31st July, 2003. One copy of the paper should be sent to Dr. Sandhya Kamath, Hon. General Secretary of API at Mumbai.

8. The decision of the panel judges will be final and binding to all concerned.

**PRESTIGIOUS AWARDS OF API**

1. GIFTED TEACHER (2003)
2. DISTINGUISHED MEMBER (2003)

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Dr. Sandhya Kamath,
Hon. General Secretary, API