Antiphospholipid antibodies in young myocardial infarction patients

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Myocardial infarction (MI) is a multi-factorial disease which claims many young lives. There are very few Indian studies that have investigated antiphospholipid antibodies (APLs) in MI patients. APLs have been implicated in arterial thrombosis including premature coronary artery and cerebrovascular thrombosis. In the present study, the prevalence of two clinically significant APLs — anticardiolipin antibody (ACA) and lupus anticoagulants (LA) in young MI patients was studied and compared with age- and sex-matched controls. Fifty healthy blood donors and 40 young MI patients (less than 45 yrs) diagnosed according to the American Heart Association guidelines were recruited for the study. The criteria for diagnosis were presence of at least two of three classical findings including: clinical symptoms, diagnostic ECG, and presence of one or more cardiac biomarkers out of raised CK-MB isoform and T-troponin on serial measurement. LA and ACA were tested by lupus-sensitive activated partial thromboplastin time (aPTT) and ELISA respectively. Elevation of ACA was observed in 9 patients, while 6 were positive for LA. ACA of IgG isotype was detected in 8 patients. One patient had LA and raised ACA of IgG and IgM isotypes. Antiphospholipid antibodies were found to be significantly associated with MI in young patients, when considered together (p<0.05) and in coronary thrombosis, mild elevation of ACA may be considered significant.

Keywords: Myocardial infarction, Anticardiolipin antibodies, Lupus anticoagulants

The incidence, prevalence, hospitalization and mortality due to coronary artery disease (CAD) in Asian Indians are 3-4 times greater than in American and European counterparts. Another serious revelation is the increased prevalence of CAD in both men and women of the younger age groups i.e., in age groups of 20-39 and 20-49 yrs respectively. While extensive research has been done on risk factors in ischemic heart disease, the role of antiphospholipid antibodies (APLs) is yet not very clearly documented and only two more reports are available from India.

Anticardiolipin antibody (ACA) and lupus anticoagulants (LA), the two most clinically significant antibodies are common cause of acquired thrombophilia. These are detected by two different systems: LA by coagulation tests and ACA by ELISA. LA is an acquired immunoglobulin (IgG, IgM or both) that interferes with phospholipid-dependent coagulation reactions, resulting in their prolongation by binding to epitopes on the phospholipid portion of prothrombinase. The three common isotypes of ACAs are IgG, IgM and IgA in order of decreasing prevalence. This study has been undertaken to examine prevalence of APLs and evaluate the significance of ACA levels in young MI patients as compared to age and sex-matched controls.

Materials and Methods

The study was undertaken at Indian Naval Hospital Ship Asvini, Mumbai, a superspeciality 800 bedded hospital in the period October 2001 to January 2004. Forty consecutive young myocardial infarction (MI) patients and 50 controls were analyzed. Inclusion criteria were (i) age <45 yrs at onset of MI (ii) absence of hypertension and diabetes mellitus and (iii) confirmed episode of MI in accordance with American Heart Association guidelines. These guidelines include presence of at least two of three classical findings: (i) clinical symptoms, (ii) diagnostic ECG and (iii) presence of one or more cardiac biomarkers out of CK-MB isoform or T-troponin on serial measurement. These two biomarkers are measures of myocardial necrosis and T-troponin is of higher diagnostic value.

Sample collection and processing

Blood samples for LA were collected in vacutainers containing 3.2% (0.109 M) trisodium citrate, centrifuged at 2500 × g for 15 min within
30 min of collection, recentrifuged at 4°C to obtain platelet poor plasma (less than $10 \times 10^9$/L) and then frozen in aliquots of 200 µl. All samples were collected from patients 10-12 weeks after MI. None of the patients were on oral anticoagulants. Plasma collected from normal healthy individuals and commercial controls both normal and abnormal were also tested along with each batch of patient samples for LA. Serum for testing for ACA was obtained from patients and controls in sterile vacutainers and again aliquots were made as above.

Sera were analyzed for β2-glycoprotein I-dependent ACA using commercial ELISA kit (OrgenTek, GmbH, Germany) with human β2-glycoprotein-I as co-factor, as per the instructions of the manufacturers. Briefly, sera were diluted 1:100, added to pre-coated plates along with reference standards provided by the manufacturer. The plates were washed, anti-human HRP conjugate added, followed by substrate addition and the subsequent color developed was measured using ELISA reader at 450 nm. A reading of IgG ACA $> 10$ IgG phospholipid (GPL units)/L and IgM ACA $> 7$ IgM phospholipid (MPL units)/L was considered positive (according to product insert and further corroborated as mean + 2 SD from values derived after testing controls).

LA was tested as described previously. A patient was considered LA positive, when the following criteria was fulfilled: (i) prolongation of coagulation time in stage I, (ii) consistent prolongation after mixing with equal vol. of normal control plasma in stage II, and (iii) inhibition of anticoagulant effect, thereby reversal to normal activated partial thromboplastin time on addition of excess phospholipid reagent. The confirmation of an abnormal result – LA or ACA positivity was in accordance with Sapporo’s criteria which requires that the abnormality should be persistent in a second sample from the patient withdrawn at least 6 weeks after the first sample. This was to exclude transient increase in these antibodies due to infections.

**Equipment**  
Clotting based tests were done using *St* art 4 (Stago Diagnostica, France), a four channel semiautomatic coagulometer which was calibrated using commercial calibrators, prior to using each new batch of kits. Complete blood count was done on hematology cell counter and ELISA based testing for ACA was done on a semi-automatic ELISA reader (Labsystems, USA). Qualitative determination of cardiac troponin-T was carried using commercial kits (Roche Diagnostics, Germany) while CK-MB was assayed on RA-50, a semi-automatic biochemistry semi-automatic analyzer (Bayer, USA) and kits from Bayer, India.

**Statistical analysis**  
Epi Info™ 6 software was used for statistical analysis of accrued data. Chi-square test was used for data analysis. The ‘$p$’ values were calculated and Yates’s correction was done, where ever applicable.

**Results**  
The age of the patients ranged from 27-45 yrs (mean age 39.2 yrs), while the mean age of control populations was 34 yrs. All controls and patients were males because MI was more frequent in male population until 7th decade, after which the incidence was similar in both sexes. Thirty-four patients (85%) had classical features, of which 8 had suffered more than one MI episode. Of the remaining 6, two each presented with unstable angina, non Q and silent MI. Smoking was commonest (39%) risk factor in patients, followed by positive family history and dyslipidemia (17.5 and 8% respectively).

APLs were detected in 14 (35%) patients, of which 9 (22.5%) were positive for ACA; six for LA (15%) including one who tested positive for both. ACA was raised in two controls (4%) and in both controls ACA was $< 13$ GPL units/L; only 4/10 patients had ACA levels $> 20$ GPL units/L with a peak value of 32 GPL units/L. The raised isotype was IgG in all patients, except one who had a concomitant raised IgM. Table 1 shows the age, number of episodes, positivity for LA, titers of ACA and associated risk factors in the 14 APL positive patients. There were only 8 patients who had more than one MI and only half of them had ACA $> 20$ GPL units/L. None of the patients had thrombocytopenia. The APL did not show any relationship to age, number of episodes or severity of MI. Presence of antiphospholipid antibodies (LA + ACA), when considered together showed significant correlation to the incidence of MI ($p = 0.04$), but not when considered individually.

**Discussion**  
LA and ACAs are two distinct entities and most of the time one occurs without the other being present especially in primary antiphospholipid antibody syndrome. ACAs have affinity to lamellar phospholipids in a bilayer composition, while LAs exhibit a stronger avidity to hexagonal phospholipids.
ACA, anticardiolipin antibodies in GPL units/L; LA, lupus anticoagulants; PL units, measurement unit for IgM; FH, positive family history; APCR, activated protein C resistance

in vitro. The levels of LA and its isotype are not important for patients, and it is adequate to detect them by using lupus sensitive aPTT and confirm their presence by the dilute Russell viper venom test.

The cut-off levels for IgG and IgM ACA have been presented in guidelines issued by Association of Clinical Pathologists, with negative results defined as <45 GPL units and <5 MPL units, low-positive results defined as values <15 GPL units and <6 MPL units, medium levels defined as 15-80 GPL units and 6-50 MPL units, and high levels defined as >80 GPL units or >50 MPL units respectively. In this study, five patients had ACA levels <20 GPL units/L and the peak value was 32 GPL units/L (Table 1). In another study (unpublished results) on bad obstetric history cases, 90% patients had ACA levels <40 GPL units/L. We, therefore, hypothesize that in Indian patients the ACA levels may not be very high. Earlier, low ACA levels were reported in another study on subjects with recurrent abortions.

This study included patients less than 45 yrs of age, as atherosclerosis is likely to be present to a lesser extent in them. Patients with other hard risk factors for MI, such as hypertension and diabetes mellitus were excluded from this study. Two patients had hypercoagulable states which could have also contributed to MI; one had low protein C and the other had activated protein C resistance. A previous study reported 60-90% concordance for ACA12 and LA; but in this study, only one sample (2.5%) showed presence of both ACA and LA. We, therefore, advocate that samples must be tested for both ACA and LA.

ACAs were present in 25% of patients which was in agreement with another study in which ACAs were detected in 22% of survivors of MI aged less than 50 yrs with two or more risk factors. In another prospective study, elevated IgG and low IgM ACA were found to contribute as independent risk factors for recurrent cardiac events, which again correlates with presence of minimally raised IgM isotype in only 1/40 patients. A limitation of this study is its retrospective nature and small number of subjects (40) enrolled. More research work is required on Indian patients with MI and these will assume greater significance if they are of prospective nature. A prospective study revealed that raised levels of ACA at 50 yrs of age correlated positively with the incidence of MI and mortality related to MI 10 to 20 yrs later. IgG and IgA ACA were associated with MI between 50 to 60 years of age and predictive power of IgA and IgG antibodies was strong and largely independent of that of other well known risk factors.

In another study, more than 20% of young (<45 yrs) survivors of acute MI harboured ACA and in those surviving, 61% patients with persistent antibodies, experienced later another thromboembolic episode. ACA probably plays a significant and major role in premature CAD; this association may approach almost 70% in young patients with CAD. An Indian study failed to show association between ACA and MI in young patients without identifiable risk factors for atherosclerosis. The role of LA in arterial thrombosis is well established, but the role of ACA is still controversial. This is in agreement with our observation that the prevalence of both LA and ACA assumed statistical significance (p = 0.04), only when considered together and not individually.

### Table 1—Profile of all myocardial infarction patients with positive antiphospholipid antibodies

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>No. of episodes</th>
<th>Age (yrs)</th>
<th>Risk factors</th>
<th>Type and titer of APL</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1</td>
<td>43</td>
<td>Smoker, triglycerides, FH</td>
<td>ACA-14</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>45</td>
<td>Smoker</td>
<td>ACA-19</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>38</td>
<td>FH: 2 relatives, ACA-20</td>
<td>APCR</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>40</td>
<td>Smoker</td>
<td>LA</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>44</td>
<td>Nil</td>
<td>ACA-23</td>
</tr>
<tr>
<td>16</td>
<td>1</td>
<td>40</td>
<td>Low protein C</td>
<td>ACA-23</td>
</tr>
<tr>
<td>17</td>
<td>1</td>
<td>40</td>
<td>Nil</td>
<td>ACA-10</td>
</tr>
<tr>
<td>18</td>
<td>2</td>
<td>43</td>
<td>Obesity</td>
<td>ACA-32</td>
</tr>
<tr>
<td>21</td>
<td>1</td>
<td>42</td>
<td>Smoker, hyperlipidemia</td>
<td>LA</td>
</tr>
<tr>
<td>24</td>
<td>1</td>
<td>30</td>
<td>Nil</td>
<td>ACA-10</td>
</tr>
<tr>
<td>26</td>
<td>1</td>
<td>41</td>
<td>FH: father died at LA 70 of MI</td>
<td>LA</td>
</tr>
<tr>
<td>28</td>
<td>1</td>
<td>36</td>
<td>Smoker</td>
<td>LA</td>
</tr>
<tr>
<td>32</td>
<td>1</td>
<td>42</td>
<td>Smoker, FH</td>
<td>LA</td>
</tr>
<tr>
<td>34</td>
<td>3</td>
<td>27</td>
<td>Nil</td>
<td>ACA IgG 10; IgM 7 MPL units: LA</td>
</tr>
</tbody>
</table>

NOTES

The study on young MI patients (40), without any other associated disease and controls (50) revealed a higher prevalence of APLs in patients, but higher levels of ACA did not correlate with multiple episodes or with their severity. APL detection will be helpful to define the patients’ risk of arterial and...
venous thrombosis and to guide therapeutic management of MI patients. More studies on prevalence of APL are required on Indian population with a larger number of controls and patients to clearly define their role in pathogenesis of MI and also for prognostication.

References
1 Enas E A, Dhawan J & Petkar S (1997) Indian Heart J 49, 25-34