SUBCLINICAL ATHEROSCLEROSIS IN PRIMARY ANTIPHOSPHOLIPID SYNDROME

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Summary

The antiphospholipid syndrome is an autoimmune disorder, which is characterized by pregnancy morbidity, recurrent arterial and/or venous thrombosis and persistently positive antiphospholipid antibodies (measured at least 12 weeks apart). The aim of this study was represented by the assessment of subclinical atherosclerosis in patients with primary antiphospholipid syndrome, using carotid artery ultrasonography and the characterization of the factors which contribute to its appearance. The study was performed on two groups of subjects: group A, formed by 10 patients with primary antiphospholipid syndrome, and group B, formed by 10 healthy sex and age-matched controls. Antiphospholipid antibodies (EIA method) were performed in patients with primary antiphospholipid syndrome, total cholesterol (Abbott photometry) was determined in all patients and control subjects. Carotid artery intima-media thickness and the presence of carotid plaques were determined using B-mode ultrasonography (ALOKA ProSound 4000, with linear transducer of 10 MHz). All the values were presented as mean ± standard deviation. The statistically analysis was done using Pearson’s test (for correlation) and Student’s t-test (for comparison), p < 0,05 was considered statistically significant. In patients with primary antiphospholipid syndrome, carotid artery intima-media thickness was increased than in controls (p < 0,001). The incidence of carotid artery plaques was greater in these patients (p < 0,05). Carotid artery intima-media thickness was correlated with antiphospholipid antibodies (r = 0,723, p< 0,01), total cholesterol (r = 0,691, p< 0,05) and age (r = 0,628, p< 0,05). The patients with primary antiphospholipid syndrome may develop premature and accelerated atherosclerosis, antiphospholipid antibodies being involved in its appearance.

Key words: primary antiphospholipid syndrome, subclinical atherosclerosis

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Introduction

The antiphospholipid syndrome (APS) was first described by Hughes G.R. in 1983, like an autoimmune disorder, which is characterized by pregnancy morbidity, recurrent arterial and/or venous thrombosis and persistently positive antiphospholipid antibodies (measured at least 12 weeks apart) (Hughes G.R., 1983). In present, this condition represents the most frequent cause of thrombophilia in adults. APS can occur as an isolated diagnosis (primary antiphospholipid syndrome) or can be associated with different diseases (especially connective tissue diseases) (Petri M., 2007). The antiphospholipid antibodies include: lupus anticoagulant, anticardiolipin antibodies (aCL), anti-β2-glycoprotein I antibodies (anti-β2GPI). These antibodies are present in about 5% of the general population, but they can occur in 50% of patients with systemic lupus erythematosus. The antiphospholipid antibodies induce thrombotic events through different cellular mechanisms (Erkan D. et al, 2009). Beyond thrombotic complications, patients with primary APS are prone to develop premature and accelerated atherosclerosis (Davies R.J. et al, 2006).
Atherosclerosis is a pathologic process, which involves the arterial walls. It is definite by the accumulation of lipid particles and different cells of the immune system in subendothelial layers, generating the thickening of the arterial walls and narrowing of the arterial lumen. Beside traditional risk factors for atherosclerosis, immune system (antibodies, antigens, autoreactive lymphocytes) is involved in this pathologic process (Hansson GK, 2005). During its evolution, atherosclerosis has an asymptomatic, subclinical stage. In this period, the diagnosis of subclinical atherosclerosis can be establish by means of carotid artery ultrasonography (Medina G., 2003).

It is demonstrated that the antiphospholipid antibodies are proatherogenic and contribute to the development of atherosclerosis. Sherer Y. et al. showed that aCL and anti-β2GPI antibodies were elevated in patients with ischemic heart disease compared with controls (Sherer Y. et al, 2001). Anticardiolipin antibodies induce monocytes adherence to endothelial cells. This process is controlled by adhesion molecules. In addition, anti-β2GPI antibodies accelerate the influx of oxLDL particles into monocytes/ macro-phages, leading to atherosclerotic plaques development (Hasunuma Y. et al, 1997).

The aim of this study was represented by the assessment of subclinical atherosclerosis in patients with primary antiphospholipid syndrome, using carotid artery ultrasonography and the characterization of APS related factors which contribute to its appearance.

**Material and methods**

The study was performed on two groups of subjects: group A, formed by 10 patients with primary APS, and group B, formed by 10 healthy sex and age-matched controls. The diagnosis of APS was established based on Revised Sapporo Classification Criteria (Miyakis S et al, 2006) for antiphospholipid syndrome.

Antiphospholipid antibodies (EIA method) were performed in patients with primary APS, total cholesterol (Abbott photometry) was determined in all patients and control subjects.

Carotid artery intima-media thickness (IMT) and carotid artery plaques were considered markers of subclinical atherosclerosis. These parameters were determined using B-mode ultrasonography (ALOKA ProSound 4000, with linear transducer of 10 MHz).

All the values were presented as mean ± standard deviation. The statistically analysis was done using Pearson’s test (for correlation) and Student’s t-test (for comparison), p < 0,05 was considered statistically significant.

**Results**

Diabetes mellitus, arterial hypertension and chronic kidney diseases were absent in all subjects. The demographic, clinical and biological characteristics of the studied groups are shown in table I.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A (APS patients)</th>
<th>Group B (controls)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males/females</td>
<td>2/8</td>
<td>2/8</td>
<td>&gt; 0,05</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41,4 ± 4,19</td>
<td>42,07 ± 3,98</td>
<td>&gt; 0,05</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>211,5 ± 20,21</td>
<td>196,2 ± 29,02</td>
<td>&gt; 0,05</td>
</tr>
<tr>
<td>aCL (GPLU/ml)</td>
<td>46,84 ± 27,94</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>30%</td>
<td>40%</td>
<td>&gt; 0,05</td>
</tr>
<tr>
<td>Carotid artery IMT (mm)</td>
<td>1,15 ± 0,14</td>
<td>0,62 ± 0,10</td>
<td>&lt; 0,001</td>
</tr>
<tr>
<td>Carotid artery plaques (%)</td>
<td>80%</td>
<td>30%</td>
<td>&lt; 0,05</td>
</tr>
</tbody>
</table>

Table I Characteristics of the studied groups
In patients with APS, carotid artery IMT was increased than in controls ($p < 0.001$). The incidence of carotid artery plaques was greater in APS patients ($p < 0.05$).

The correlations between IMT and aCL, total cholesterol and age are presented in table II and figure 4.

**Table II** The correlations between IMT and aCL, age, cholesterol

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCL</td>
<td>0.723</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.691</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>age</td>
<td>0.628</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

**Discussion**

The antiphospholipid syndrome represents the most important acquired hypercoagulable state. It is definite by the arterial and/or venous thrombosis, pregnancy morbidity, in the setting of antiphospholipid antibodies (confirmed twice over at least 12 weeks) (Hughes G.R., 1983). Revised Sapporo Classification Criteria for antiphospholipid syndrome are listed in table III (Erkan D. *et al*, 2009).
Table III Revised Sapporo Classification Criteria

| Clinical criteria | - Vascular thrombosis  
|                  |   • one or more clinical episodes of arterial, venous, or small vessel thrombosis in any tissue or organ  
|                  |   - Pregnancy morbidity  
|                  |   • one or more unexplained fetal death without any morphological abnormalities at or beyond the 10th week of gestation, or  
|                  |   • one or more premature birth of a morphologically normal neonate before 34th week of pregnancy due to severe preeclampsia, eclampsia or placental insufficiency, or  
|                  |   • three or more first trimester losses in the absence of maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes  
| Laboratory criteria | - Lupus anticoagulant  
|                | - Anticardiolipin antibodies  
|                | - Anti-β2-glycoprotein I antibodies (on two occasions 12 weeks apart)  

The antiphospholipid syndrome is definite by the presence of at least one of the clinical criteria and one of the laboratory criteria.

Other factors, which are not included in these criteria, may be helpful in the diagnosis of APS. They are represented by: livedo reticularis, thrombocytopenia, autoimmune hemolytic anemia, valvular heart disease, early preeclampsia, IgA anticardiolipin antibodies, IgA anti-β2-glycoprotein I antibodies (Petri M., 2007; Erkan D. et al, 2009).

Antiphospholipid antibodies represent a family of autoantibodies directed against phospholipid-binding plasma proteins. Because these phospholipids contribute to control of coagulation, this family of autoantibodies can lead to a hypercoagulable state. Antiphospholipid antibodies include: lupus anticoagulant, anticardiolipin antibodies and anti-β2-glycoprotein I antibodies (Hughes G.R., 1983).

The patients with primary APS are prone to develop accelerated atherosclerosis (Davies R.J. et al, 2006). Kaplan S.D. reported coronary artery disease and coronary artery bypass occlusion in patients with APS (Kaplan S.D. et al, 1992). In his study, Sherer Y showed that the patients with severe ischemic heart disease had elevated levels of aCL, anti-β2GPI, anti-oxLDL antibodies compared with those without significant coronary artery stenosis (Sherer Y. et al, 2001).

Beyond traditional risk factors, in the initiation and progression of atherosclerosis, inflammatory and immunogenic mechanisms are involved. Oxidation of low-density lipoproteins (LDL) leads to oxidized LDL (oxLDL) particles. Anti-β2GPI antibodies accelerate the influx of LDL particles in monocytes/macrophages. In atherosclerotic specimens obtained from human carotid endarterectomies, oxLDL is detected co-localized with β2GPI and lymphocytes. β2GPI (apolipoprotein H) is found in the subendothelial regions and in the intima-media layers, at the border of human atherosclerotic plaques, representing the target for an autoimmune reaction, that could promote lesion initiation and progression (Hasunuma Y et al, 1997; Steinberg D., 1997; George J. et al, 1999).

OxLDL binds β2GPI, resulting oxLDL/β2GPI complexes. These com-

Carotid intima-media thickness is a sensitive marker for the earliest stages of atherosclerosis in APS. Charakida M. et al. showed that in patients with APS, carotid artery IMT was increased compared to controls (0.75 ± 0.02 mm versus 0.64 ± 0.01 mm, p < 0.001) (Charakida M. et al, 2006). The same results were obtained by Medina G et al in patients with primary APS, with the mean age of 40 years (Medina G et al, 2003). In our study, carotid artery IMT in patients with primary APS was increased than in controls (1.15 ± 0.14 mm versus 0.62 ± 0.10 mm, p < 0.001). Carotid artery IMT was correlated with the values of aCL (r = 0.723, p < 0.01), total cholesterol (r = 0.691, p < 0.05), and age (r = 0.628, p < 0.05). The incidence of carotid artery plaques was greater in APS patients (p < 0.05). The strong correlation between carotid artery IMT and aCL demonstrated the role of these antibodies in the pathogenesis of atherosclerosis in primary APS patients.

**Conclusion**

The patients with primary APS develop premature and accelerated atherosclerosis, antiphospholipid antibodies being involved in its appearance.

**References**


