Anti-Cardiolipin and Anti-Neutrophil Cytoplasmic Antibodies in Iranian Patients with Behcet’s Disease

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ABSTRACT

Background: The prevalence of anti-Neutrophil Cytoplasmic Antibodies (ANCAs) and anti-Cardiolipin Antibodies (anti-CL Ab) in Behcet’s Disease (BD) and also their roles in vascular involvement is controversial. Objective: To assess the prevalence of ANCAs and anti-CL Ab as well as their correlations with clinical manifestations in Iranian patients with BD. Methods: In this case/control study, the sera from 88 patients with BD and 88 healthy controls were evaluated. The levels of ANCAs and anti-CL Ab were measured using indirect ELISA method. Results: The levels of anti-CL, anti-PR3 and anti-MPO (Myeloperoxidase) IgG autoantibodies between BD patients and healthy controls were not statistically different (p=0.21, p=0.28 and p=0.74, respectively). In addition, there were no significant differences between BD patients with and without vascular involvement in the levels of anti-CL (1.42 ± 1.24 GPLU/ml and 1.58 ± 1.18 GPLU/ml, respectively; p=0.71), anti-PR3 (0.0 ± 0.0 U/ml and 0.08 ± 0.27 U/ml, respectively; p=0.10) and anti-MPO (0.48 ± 0.23 U/ml and 0.52 ± 0.22 U/ml, respectively; p=0.41) IgG autoantibodies. Nevertheless, mean titer of anti-CL IgG was higher in male patients with skin rash than those without skin rash (2.2 ± 0.88 GPLU/ml and 1.11 ± 1.22 GPLU/ml, respectively; p=0.017). Conclusion: While anti-CL, anti-PR3 and anti-MPO IgG autoantibodies do not play a major role in susceptibility to BD or pathogenesis of vascular involvement in our patients, anti-CL Ab might be involved in skin lesion development in Iranian male BD patients. However, the results should be confirmed in other studies.


Keywords: ANCA, Anticardiolipin Antibody, Behcet’s Disease

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INTRODUCTION

Behcet's disease (BD) or Silk-road disease is an immune-mediated inflammatory disorder with systemic vasculitis (1). Mucous membrane ulceration and ocular involvements are among the most important clinical manifestations in patients with BD. Small-vessel vasculitis is thought to underlie much of the pathological process of BD, and clinically evident large-vessel involvement occurs in 7-49% of patients (2,3). The vasculitis of BD is distinctive because of involvement of both arteries and veins of all sizes. Venous vascular involvement is one of the most unique features of vasculitis in BD (4) and is thought to be responsible for thrombosis in these patients (5). Although the pathological mechanisms of vasculitis and subsequent thrombosis in BD have not been well known, involvement of some autoimmune mechanisms that target the endothelial cells have been suggested (6-8). In addition, associations between the existence of anti-neutrophil cytoplasmic antibodies (ANCAs) directed at the azurophilic granule proteins (proteinase-3 and myeloperoxidase) and various diseases with small-vessel vasculitis such as Wegner granulomatosis (9), microscopic polyangiitis (10) and Churg-Strauss syndrome (11) have been reported. Interestingly, ANCAs have been found to be positive in 8 out of 66 Turkish patients with BD (10.2 %) by combination testing consisting of immunofluorescence and ELISA (12). The frequency of ANCAs positivity in patients with BD is approximated to the reported prevalence of vascular involvement (14.3%) in Turkish patients with BD (13). Therefore, ANCAs may play an important role in vascular lesions in BD.

Regarding thrombosis, while there has been no consistent primary abnormality of the coagulation, anticoagulation, or fibrinolitic systems identified in BD patients (14), factor V Leiden, HLA-B5 (B51) and MEFV mutations have been found to be risk factors of thrombophlebitis in certain ethnic groups (15). Besides, association between the presence of anti-cardiolipin antibody (anti-CL Ab) and arterial and venous thrombosis in various diseases has been detected (16,17). Since the thrombosis and vascular damage are prominent in BD, various studies have focused on assessment of anti-CL Ab in BD (18). Although in the most of previous studies the frequency of anti-CL Ab in BD was very low (18,19), some studies have reported the presence of anti-CL Ab in approximately 50% of patients with BD (20).

The complexity of susceptibility factors as well as ethnic and geographical differences complicates attempts to implement the above mentioned findings in diverse populations, therefore in the present study the prevalence of ANCAs (anti-PR3 and anti-MPO Abs) and anti-CL Ab in Iranian patients with BD and also their correlations with vascular involvement and other clinical manifestations were investigated.

MATERIALS AND METHODS

Subjects. After obtaining the informed consent from all participants, sixty six BD patients (n=66; 28 males and 38 females, mean age 35.4 ± 9.3 years) who fulfilled the International Study Group (ISG) for BD criteria (21) were randomly selected for this case/control study. From these patients, twenty-two BD patients had no history of immunosuppressive therapy before sampling. Among the sixty six BD patients, only two patients have vascular involvement. Therefore for investigation of the association between the vascular involvement and anti-PR3 or anti-CL Ab positivity, the sera and
clinical data of twenty two BD patients with vascular involvement from the biologic bank of Autoimmune Diseases Research Center (Shiraz University of Medical Sciences) were also included in this study. Thrombosis was the only kind of vascular involvement in this group of patients. Skin involvement was determined by clinical observation of skin and in some cases by histopathologic examination of skin biopsy. Patients showed two types of skin lesions, erythema nodosum like and papulopustular lesions. In the group of 66 randomly selected patients with BD, five patients had erythema nodosum like lesions and 21 patients had papulopustular lesions while in 22 patients who were selected from biologic bank of autoimmune diseases research center, two and eight patients represented with erythema nodosum like and papulopustular lesions, respectively. The involvement of joint was determined by clinical examination by rheumatologist. For diagnosis of vascular involvement, Color Doppler Sonography was done in clinically suspicious patients. Moreover, the determination of retinal vascular disease was made by intravenous fluorescein angiography in selected cases by ophthalmologist.

Eighty-eight healthy volunteers (n=88; 45 males and 43 females, mean age 38.2 ± 10.3 years) without any history and any sign of autoimmune diseases in physical exam were also recruited as normal controls. The control group was selected by age and sex pair wise matching with control group. The mean levels and frequencies of anti-PR3, anti-MPO and anti-CL Abs were not different between patients with and without active disease.

**ELISA Assay.** All collected sera were kept at -80°C until analysis. The sera were tested for the presence of IgG anti-MPO (Genesis Diagnostic, Cambridge, UK), IgG anti-PR3 and IgG anti-CL antibodies (Orgentec Diagnostika GmbH, Mainz, Germany) by quantitative indirect ELISA assays according to the manufacturers' recommendations. Cut off points of 10 GPL U/ml, 5 U/ml and 4 U/ml were considered for IgG anti-CL, IgG anti-PR3 and IgG anti-MPO positivity, respectively. The levels of Abs were presented as mean ± SD.

### Table 1. Demographic and clinical features of BD patients.

<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>Frequency</th>
<th>Age</th>
<th>Age at disease onset</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>(Mean year ± SD)</td>
<td>(Mean year ± SD)</td>
</tr>
<tr>
<td>Oral ulcers</td>
<td>63 (95)</td>
<td>34.7 ± 8.9</td>
<td>27.4 ± 8.5</td>
</tr>
<tr>
<td>Genital ulcers</td>
<td>43 (65)</td>
<td>35.3 ± 8.6</td>
<td>27.9 ± 9.1</td>
</tr>
<tr>
<td>Vascular involvement</td>
<td>2 (3)</td>
<td>42.5 ± 9.1</td>
<td>37.5 ± 4.9</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>3 (4)</td>
<td>37.6 ± 12</td>
<td>31.6 ± 10.6</td>
</tr>
<tr>
<td>Skin involvement</td>
<td>36 (54)</td>
<td>33.3 ± 8.5</td>
<td>27.3 ± 9.1</td>
</tr>
<tr>
<td>Eye involvement</td>
<td>16 (24)</td>
<td>35.8 ± 8.7</td>
<td>27.7 ± 9.1</td>
</tr>
<tr>
<td>Joint involvement</td>
<td>42 (63)</td>
<td>34.7 ± 9.8</td>
<td>26.6 ± 9</td>
</tr>
<tr>
<td>CNS involvement</td>
<td>26 (39)</td>
<td>34.7 ± 10.1</td>
<td>26.8 ± 8</td>
</tr>
</tbody>
</table>
**Statistical Analysis.** The normality of data distribution was investigated with Kolmogorov-Smirnov test (K-S test). The non-parametric Mann-Whitney U-test was used to compare anti-MPO, anti-PR3 and anti-CL Ab levels between BD patients and normal group or BD patients with and without specific clinical symptoms. The chi square test was used to test the association of anti-MPO, anti-PR3 and anti-CL Ab positivity and susceptibility to BD and also their associations with various clinical manifestations. The statistical significance level was set at 0.05.

**RESULTS**

The frequencies of different clinical manifestations in the group of 66 BD patients after classification of patients according to their demographic features are demonstrated in Table 1. As shown in Table 2 and Table 3, the levels of ANCAs (anti-PR3 and anti-MPO IgG autoantibodies) showed no statistically significant difference between BD patients and healthy controls.

**Table 2. Anti-PR3, anti-MPO and anti-CL Abs levels (Mean ± SD) in the group of 66 randomly selected BD patients and normal group.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>BD (n=66)</th>
<th>Healthy controls (n=88)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG anti-PR3 Level (U/ml)</td>
<td>0.35 ± 1.18</td>
<td>0.22 ± 0.94</td>
<td>0.28</td>
</tr>
<tr>
<td>IgG anti-MPO Level (U/ml)</td>
<td>0.51 ± 0.22</td>
<td>0.51 ± 0.27</td>
<td>0.74</td>
</tr>
<tr>
<td>IgG anti-CL Level (GPL U/ml)</td>
<td>1.55 ± 1.19</td>
<td>1.24 ± 1.18</td>
<td>0.21</td>
</tr>
</tbody>
</table>

In addition, there was no significant difference in the levels of IgG anti-PR3 Ab or anti-MPO Ab between BD patients (total patients, males and females) with and without different clinical manifestations (data not shown). Moreover, no association was found between the presence of IgG anti-PR3 Ab or anti-MPO Ab and vascular involvement or other clinical manifestations in BD patients.

**Table 3. Anti-PR3, anti-MPO and anti-CL Abs levels (Mean ± SD) in total 88 BD patients (66 randomly selected and 22 BD patients with documented vascular involvement) and normal group.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>BD (n=88)</th>
<th>Healthy controls (n=88)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG anti-PR3 Level (U/ml)</td>
<td>0.26 ± 1.0</td>
<td>0.22 ± 0.94</td>
<td>0.73</td>
</tr>
<tr>
<td>IgG anti-MPO Level (U/ml)</td>
<td>0.53 ± 0.27</td>
<td>0.51 ± 0.27</td>
<td>0.8</td>
</tr>
<tr>
<td>IgG anti-CL Level (GPL U/ml)</td>
<td>1.50 ± 1.20</td>
<td>1.24 ± 1.18</td>
<td>0.25</td>
</tr>
</tbody>
</table>
Also, comparison of anti-CL IgG Ab levels between patients and controls did not show a significant difference (1.5 ± 1.2 GPL U/ml and 1.26 ± 1.18 GPL U/ml, respectively; p=0.21; Table 3). On the other hand, similar to ANCAs, no association was found between the presence of IgG anti-CL Ab and different clinical manifestations, including vascular involvement, in BD patients. However, after categorization of patients according to their gender, the mean level of IgG anti-CL Ab was higher in male patients with skin lesions than those without skin involvement (2.2 ± 0.88 GPL U/ml and 1.11 ± 1.22 GPL U/ml, respectively; p=0.017). The mean level and frequency of anti-PR3, anti-MPO and anti-CL Abs were not significantly different between 22 patients with vascular involvement and controls.

**DISCUSSION**

The aim of the present study was to investigate the prevalence of ANCAs (anti-PR3 and anti-MPO Abs) and anti-CL Ab in Iranian patients with BD and their association with clinical manifestations of BD in our set of patients. Therefore, the frequencies of Iranian BD patients with positive ANCAs (anti-PR3 and anti-MPO IgG Abs) and anti-CL IgG Ab were investigated in the present study and the levels of these autoantibodies were compared with the age- and sex-matched controls. The results showed that the levels and positivity of anti-PR3 and anti-MPO IgG were not significantly different between BD patients and healthy controls. In fact, the frequency of patients and controls with positive anti-PR3 IgG were 2.27% and 0%, respectively (p=0.49), while none of the patients and controls were found to be positive for anti-MPO. In this respect, our results are consistent with reports of previous investigators who showed the lack of or insignificant presence of ANCAs positive cases among BD patients (22,23). In addition, it has been reported that BD patients with vascular involvement are usually not positive for anti-PR3 and anti-MPO Abs (23). Similarly, in the present study no significant association was observed between the presence of both clinically important ANCAs and different clinical manifestations of BD, including vascular involvement, in a larger number (n=88) of Iranian patients with BD.

Published data suggest that the frequency of anti-CL IgG cases among patients suffering from BD is very different (18,21,24). The results of the present study showed that the frequency of anti-CL IgG positivity in Iranian patients with BD is equal to 3.4%. Concerning anti-CL IgG Ab, our data is consistent with many of previous studies that reported 0-8% of BD patients having anti-CL Abs (18). In this respect, Tokay et al. reported only 0.8% anti-CL IgG Ab positivity in 128 Turkish patients with BD (18). However, the frequency of BD patients with anti-CL IgG Ab were reported to be 40-50% in countries such as Germany and Saudi Arabia (21,25). These differences may be explained by geographical varieties whether in genetic or environmental factors, that may affect not only the frequency of anti-CL Ab but also the clinical manifestations of BD (18,26). The results of the present study also showed that the mean levels of anti-CL IgG as well as the frequency of patients with positive anti-CL Ab were not significantly different from normal controls (p=0.25 and p=0.24, respectively). Moreover, we categorized the patients according to their gender. In the male patients with skin involvement, the mean titer of anti-CL IgG was significantly higher than those without skin lesions. This finding is interesting since the link between anti-CL Abs and skin diseases has been poorly addressed in medical literature (8). Therefore, the possible link
between BD and anti-CL Abs warrants more investigations for the role of anti-CL Abs in skin manifestations as well as immunopathology of skin lesions in BD patients. In conclusion, it seems that anti-PR3, anti-MPO and anti-CL Abs neither play an important role in BD development nor are important in thrombosis and vascular involvement in Iranian BD patients. However, the titer of anti-CL IgG Ab is significantly higher in male BD patients with skin involvement compared to those without skin manifestations. Assessment of other classes of the above mentioned autoantibodies (especially IgM and IgA) might add valuable data to our understanding of BD pathogenesis.

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REFERENCES


