Anti-Cyclic Citrullinated Peptide Antibodies in Rheumatoid Arthritis and Behçet’s Disease

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Rheumatoid arthritis (RA) is associated with progressive joint destruction and disability. Early diagnosis of RA is important, since early and aggressive treatments lead to a better outcome. Circulating autoantibodies are a serological hallmark of systemic rheumatic diseases. More recently, antibodies directed to a cyclic citrullinated peptide, anti-CCP antibodies, have been established as specific diagnostic and prognostic tools for RA. The aims of this study were to assess the diagnostic and radiological prognostic value of the anti-CCP antibody in Turkish patients with RA (n = 97) and those with Behçet’s disease (BD) (n = 46). The study also included 35 healthy controls. Anti-CCP antibodies were measured by ELISA, and radiological damage was evaluated by using modified Larsen scoring. In the RA group, sensitivity and specificity were 80.4% and 93.5% for rheumatoid factor (RF), and 74.2% and 97.8% for anti-CCP antibody, respectively. RF was positive in 3 BD patients (6.5%) and in one of the controls (2.9%). In contrast, anti-CCP antibodies were detected in one BD patient (2.2%), but not in the control subjects. Deformed joint counts and radiographic scores were higher in anti-CCP antibody-positive RA patients (n = 72) than those in anti-CCP antibody-negative patients (n = 25) (p < 0.05). Moreover, anti-CCP antibody titer correlated with deformed joint count (r = 0.224, p < 0.05) and radiographic score (r = 0.308, p < 0.05). This study indicates the diagnostic and prognostic utility of anti-CCP antibodies in Turkish patients with RA. Importantly, anti-CCP antibodies are not associated with BD.

Rheumatoid arthritis (RA) is a chronic autoimmune disease and affects approximately 1% of the world population (Fox 2005), causing persistent synovitis, pain, joint destruction and functional disability. The emphasis in the management of RA is early diagnosis and effective intervention. Irreversible joint destruction can be prevented by the treatment during the first months of the disease (O’Dell 2002; Bukhari et al. 2003); early and accurate diagnosis is critical in the RA management.

Rheumatoid factor (RF), an antibody direct-
ed against the Fc region of IgG, has been used as a diagnostic marker for RA (Arnett et al. 1988; Fox 2005). However, although RF has little diagnostic utility, its place has been retained in practice because of its prognostic ability and the lack of alternative tests (Quinn et al. 2005). It is known as a nonspecific marker, since it may also be detected in patients with other autoimmune and infectious diseases and even in healthy persons.

Anti-citrullinated peptide antibodies, including anti-perinuclear factor (APF), anti-keratin and anti-filagrin antibodies (AKA, AFA), are described as to be the factors of a highly specific antibody system for RA (Nienhuis and Mandema 1964). However, APF and AKA have never been widely used as the markers for RA because of technical limitations. Sensitive assays having easier measurement methods and using a cyclic-citrullinated peptide (CCP) have been developed to detect anti-CCP antibodies (Schellekens et al. 2000). Anti-CCP antibodies particularly bind to antigenic determinants which contain amino acid, citrulline that is formed by a post-translational modification of arginine residues by the enzyme peptidyl arginine deiminase (Schellekens et al. 2000).

Anti-CCP antibodies have been proved to be an efficient diagnostic marker for RA, as they can be detected with high disease specificity in more than 80% of serum samples taken from RA patients (Suzuki et al. 2003). Moreover, anti-CCP antibodies are now considered as important markers for the diagnosis of RA and as probable prognostic markers for the development of erosive arthritis (van der Helm-van Mil et al. 2005; Syversen et al. 2007).

In several studies the diagnostic value of anti-CCP antibodies has been assessed and confirmed that they are an indicator or predictor of the prognosis in RA (Lee and Schur 2003; Sanmarti et al. 2007). However, as genetic differences and environmental factors may alter the anti-CCP antibody positivity (van Gaalen et al. 2004; Furuya et al. 2007; Kokkonen et al. 2007; Verpoort et al. 2007), the aims of the present study were directed to evaluate the frequency of anti-CCP antibody positivity in Turkish patients with RA and those with Behçet’s disease (BD) and to determine its relation with the clinical and radiological severities of RA in our cohort of RA patients.

**PARTICIPANTS AND METHODS**

The study enrolled 97 patients with RA (mean age 47.7 ± 12.2 years; 18 males, 79 females) and 46 patients with BD (mean age 34.9 ± 8.9 years; 24 males, 22 females) and 35 healthy controls (HC) (mean age 36.2 ± 10.7 years; 18 males, 17 females). Patients with RA or BD were diagnosed according to the established diagnostic criteria of the American College of Rheumatology (ACR) (Arnett et al. 1988) or the International Study Group for BD (ISG 1992), respectively. All the participants included in the study were informed about the study, and their consents were taken. Detailed histories of all the participants were obtained, and their systemic and rheumatologic examinations were performed. Disease activity in the RA group was determined in accordance with the disease activity score-28 (DAS-28) (Leeb et al. 2005).

In the RA group, standard radiographs of the hands and wrists were obtained (blood samples and X-ray films were taken on the same day), and all radiological features were evaluated by the same radiologist who was blinded to the clinical and serological data of the patients. The modified Larsen method was used to evaluate radiographic damage; twenty joints in the hands and two wrists (each wrist was considered as a unit) were assessed with the scores ranging from 0 to 5, as described (Zangger et al. 2004).

Laboratory examinations were performed on blood samples from the participants who had fasted overnight. Conventional biochemical values were studied using Olympus kits (Olympus Corp., Tokyo) in an Olympus AU 600 Autoanalyzer by routine clinical methods. Erythrocyte sedimentation rate (ESR) was determined by the Westergren method, and the level of C-reactive protein (CRP) and the titer of RF were measured by the immunoturbidimetric technique (Schiapparelli Biosystems, Woerden, the Netherlands) and nephelometric technique (BNII, Dade Behring, Marburg, Germany), respectively. IgM-RF titers higher than 15 IU/ml were considered as positive. Blood samples (5 ml) were obtained for anti-CCP antibody analysis. The samples were centrifuged at 3,000 rpm for 10 min to obtain sera, and they were stored at −20°C until the day of the analyses.
Stored sera were tested for anti-CCP antibodies by ELISA using a second-generation commercial assay kit (Euroimmun, Lubeck, Germany). The ELISA was carried out according to the manufacturer’s instructions with a cut-off value of 5.01 units.

**Statistics**

Statistical analysis was performed using the Statistical Package for the Social Sciences version 11.0 (SPSS, Chicago, IL, USA). One-way analysis of variance (ANOVA) followed by the Scheffe post hoc test, and for dual comparisons the Student t-test, were used. Correlations were assessed using the Pearson product moment test. Odds ratio (OR) were calculated, with 95% confidence interval (CI), and the chi-square test was used to compare categorical variables. Results were given as means, and standard deviations. Differences were considered as significant at $p < 0.05$.

**RESULTS**

The demographics and laboratory data pertaining to all three groups are presented in the Table 1. IgM-RF and anti-CCP antibodies were detected in the sera as 80.4% ($n = 78$) and 74.2% ($n = 72$) in the RA group, respectively. In the BD and HC groups, frequencies of IgM-RF positivity were 6.5% ($n = 3$) and 2.9% ($n = 1$), respectively, and anti-CCP positivity was found as 2.2% ($n = 1$) in the BD group while none of the HC was anti-CCP-positive.

In the RA group, sensitivity and specificity were 80.4% and 93.5% for IgM-RF, and 74.2% and 97.8% for anti-CCP antibody, respectively. Five (26.3%) out of 19 RF-negative RA patients had anti-CCP antibody positivity; 11 (44%) out of 25 anti-CCP antibody-negative RA patients had RF positivity. Thus, anti-CCP antibody predicted RA in 26.3% of RF-negative patients. The presence of RF or anti-CCP antibody increased the sensitivity to 85.6% ($n = 83$), but did not improve specificity, since a BD patient with anti-CCP antibody-positive was also RF-positive.

Deformed joint counts and modified Larsen scores were high in the anti-CCP antibody-positive RA group, when compared with anti-CCP antibody-negative group ($p < 0.05$ for both, Table 2). In the RA group, anti-CCP antibody titer positively correlated with IgM-RF titer ($r = 0.344, p < 0.001$), deformed joint count ($r = 0.224, p < 0.05$).

**Table 1.** Demographic and laboratory data of the RA, BD and HC groups.

<table>
<thead>
<tr>
<th></th>
<th>RA ($n = 97$)</th>
<th>BD ($n = 46$)</th>
<th>HC ($n = 35$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47.7 ± 12.2*</td>
<td>34.9 ± 8.9</td>
<td>36.2 ± 10.7</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>18/79*</td>
<td>24/22</td>
<td>18/17</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>5.6 ± 6.3†</td>
<td>2.5 ± 4.3</td>
<td>-</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>7.3 ± 0.6*</td>
<td>7.5 ± 0.6</td>
<td>7.6 ± 0.5</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.1 ± 0.6*</td>
<td>4.3 ± 0.4*</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td>WBC ($10^3/μl$)</td>
<td>9.4 ± 3.3*</td>
<td>9.3 ± 3.1*</td>
<td>7.2 ± 1.4</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12.8 ± 1.8*</td>
<td>13.6 ± 1.9†</td>
<td>14.7 ± 1.9</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>40.5 ± 31.6*</td>
<td>21.9 ± 18.5†</td>
<td>11.1 ± 10.8</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>34.8 ± 42.7*</td>
<td>18.7 ± 26.6*</td>
<td>4.2 ± 2.9</td>
</tr>
<tr>
<td>RF positivity (%)</td>
<td>80.4*</td>
<td>6.5</td>
<td>2.9</td>
</tr>
<tr>
<td>Anti-CCP positivity (%)</td>
<td>74.2*</td>
<td>2.2</td>
<td>0</td>
</tr>
</tbody>
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RA, rheumatoid arthritis; BD, Behçet’s disease; HC, healthy control; M, male; F, female; WBC, white blood cell count; Hb, haemoglobin; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF, rheumatoid factor; anti-CCP, anti-cyclic citrullinated peptide antibody.

vs the C group; $p < 0.001*$, $p < 0.01$†.

vs the BD group; $p < 0.001*$, $p < 0.05$‡.

Odds ratio; 54.1 (95% confidence interval 7.7 to 379.9) for anti-CCP antibody positivity and 14.1 (95% confidence interval 5.1 to 36.8) for RF positivity.
and modified Larsen score \((r = 0.308, p < 0.05)\), and titer of IgM-RF also positively correlated with modified Larsen score \((r = 259, p < 0.05)\) and CRP level \((r = 308, p < 0.01)\). A trend towards longer disease duration at the time of admission was observed for anti-CCP antibody-positive patients compared with -negative patients \((p = 0.217)\), and therefore additional logistic regression analyses were performed to correct for the disease duration. Anti-CCP antibodies were still found to be a significant predictor of the modified Larsen score \((p < 0.05)\).

The modified Larsen score \((p < 0.01)\) and ESR level \((p < 0.05)\) were higher in the female patients compared with the male ones, in the RA group, although anti-CCP antibodies and RF positivity were not different. The modified Larsen score of female gender was higher than that of the male gender in both anti-CCP antibody-positive and -negative subgroups \((p < 0.05\) for both).

Twenty three patients of the RA group were newly diagnosed, and they were not receiving disease-modifying antirheumatic drugs or corticosteroids. Anti-CCP antibody and RF were positive in 16 (69.6%) and 17 (73.9%) of the recently diagnosed patients, and 56 (75.7%) and 61 (82.4%) of the established patients, respectively. DAS-28 scores were 4.4 ± 0.9 and 3.6 ± 1.3 in the recently diagnosed and established RA patients, respectively, being significantly higher in the former group \((p < 0.05)\). However, deformed joint counts and modified Larsen scores were higher in the established RA patients than those in the recently diagnosed patients \((p < 0.05, p = 0.001, \text{respectively})\). Deformed joint counts were 4.3 ± 7.4 and 1.1 ± 3.6, and modified Larsen scores were 16.1 ± 3 and 3.1 ± 7.7 in the established and recently diagnosed RA groups, respectively.

Ten had anti-CCP antibodies, and 9 had RF positivity in the 11 smoker RA patients (9 current and 2 former smokers). Smoking increased the frequencies of anti-CCP antibody and RF positivity with an odds ratio of 3.8 (95% CI 0.3 to 25.1) for anti-CCP antibody and 2.9 (95% CI 0.3 to 25.1) for RF \((p > 0.05\) for both).

**DISCUSSION**

RA does not directly threaten life, but severely affects the quality of life of a patient and also has major economical consequences for the society since it may cause progressive joint destruction and disability. Moreover, early diagnosis of
RA is important, as irreversible joint destruction can be prevented by intervention during the early period of the disease (O’Dell 2002; Bukhari et al. 2003). Although RF is still the unique serological criterion of ACR criteria set (Arnett et al. 1988), it is neither highly sensitive nor specific for the diagnosis of early RA (Goldbach-Mansky et al. 2000). It has relatively low specificity due to being sometimes detected in patients with other autoimmune diseases and unaffected healthy people. In contrast, anti-CCP antibodies, which have higher specificity than RF and comparable sensitivity with that of RF (De Rycke et al. 2004), are good serologic markers for RA (Suzuki et al. 2003; De Rycke et al. 2004). Our study confirms that anti-CCP antibody is more predictive than RF in RA patients.

More reliable predictors of joint damage for RA are still to be discovered. Moreover, the current therapeutic strategies using aggressive regimens prevent disability and loss of quality of life, but they may unfortunately be harmful. Anti-CCP antibodies which are thought to be directly involved in the pathogenesis of RA (Vossenaar et al. 2004) are now considered as important prognostic markers for the development of erosive RA (van der Helm-van Mil et al. 2005). Additionally, early RA patients with anti-CCP antibody-positivity have been reported to have more severe radiological damage than patients without this antibody during follow-up (Kroot et al. 2000; van der Helm-van Mil et al. 2005). In our study, anti-CCP antibody-positive RA patients had also higher radiographic score and deformed joint count compared to anti-CCP-negative patients, and the titer of anti-CCP antibodies also positively correlated with radiographic score, and finally these suggested that anti-CCP antibody might reflect the radiological progression.

Previous studies have reported anti-CCP antibody positivities in about 34-60% of RF-negative RA patients (Lee and Schur 2003; Quinn et al. 2005). Anti-CCP antibodies were positive in 26.3% of RF-negative RA patients in our study. Genetic differences may be the reason for this relatively lower prevalence. The protein tyrosine phosphatase (PTPN22) 1858C/T polymorphism has been reported to be associated with anti-CCP antibody positivity in early RA in a study from Northern Sweden (Kokkonen et al. 2007). However, preliminary data from a recent study from Turkey has shown that PTPN22 polymorphism is not related with RA (the data presented in the 2007 Annual Turkish National Rheumatology Congress). Furthermore, in Northern European Caucasian and Japanese patients with RA, anti-CCP antibodies have been found as associated with the human leukocyte antigen (HLA)-DRB1 shared epitope (SE) alleles (van Gaalen et al. 2004; Furuya et al. 2007). However, SE was detected in 70.2% of Turkish patients with RA (Saruhan-Direskeneli 1998).

Prevalence of anti-CCP antibody positivity has been reported to be as 77% for established and 60% for early RA (van Venrooij et al. 2006). However, the presence of anti-CCP antibodies was relatively higher in the recently diagnosed RA patients in our study. The relatively higher percentage of anti-CCP antibody positivity among recently diagnosed RA group may be explained by the delay in RA diagnosis since our RA patients encompass the full range of RA severity/activity even in the recently diagnosed RA patients, due to a small number of rheumatologists and due to the Health system in Turkey. Our hospital accepts referrals from primary and secondary health centers, and thus patients with more severe RA might form our patient group. Relatively higher deformed joint counts and modified Larsen scores for newly diagnosed patient are also evidence for this delay.

Smoking is one of the environmental susceptibility factors for RA although the precise mechanisms of this susceptibility are still required to be documented. Cells from bronchoalveolar lavage of smokers have been reported to express higher levels of citrullinated antigens, compared to those from non-smokers (Klareskog et al. 2006). Smoking has also been shown to be a risk factor for the development of anti-CCP antibody-positive RA (Klareskog et al. 2006), and higher anti-CCP antibody positivity has been reported in smoker RA patients than in non-smoker ones (Verpoort et al. 2007). Smoking has been shown
to be related with anti-CCP positivity in both SE-positive and -negative RA patients (Verpoort et al. 2007). In our study, anti-CCP antibody was positive in 90.1% of smoker RA patients, while in only 72.1% of non-smoker ones. In cross-sectional studies, smokers have been reported to have more erosions on radiographs (Saag et al. 1997). Increased titers of anti-CCP antibodies might be the cause of this poor prognosis in the smoker patients.

Positivity of RF, anti-CCP antibody and SE, disease duration and female gender are known as important predictors of radiographic progression in RA (Sanmarti et al. 2007). In our study, modified Larsen score of female gender was higher than that of male gender in both anti-CCP antibody-positive and -negative subgroups.

There has been no adequate number of publications about anti-CCP antibody in BD although it has been studied widely in autoimmune diseases with joint involvement other than RA (Lee & Schur 2003). BD is a frequent inflammatory disease in our country and may also affect joints. Therefore patients with BD were included in this study as the patient control group for comparison. One out of 3 (Kwok et al. 2005) and one out of 5 (Sghiri et al. 2007) BD patients have been reported to be anti-CCP antibody-positive. However, in another study (Kogure et al. 2007) none of 4 BD patients has been anti-CCP antibody-positive. One patient from our BD group was anti-CCP antibody and IgM-RF positive. She had previously received interferon, cyclosporine, sulphasalazine and corticosteroid for uveitis (diagnosed 20 years ago) and BD (diagnosed 2 years ago with fulfilled criteria of BD). Moreover, she was smoker for 13 years. Results of our study suggest that BD may not be associated with anti-CCP antibodies.

Relatively small sample size and absence of determining the relationship between SE allele carriage and anti-CCP antibody positivity might be the limitations of our study. Moreover, treatments may alter anti-CCP titer. Rönnelid et al. (2005) have shown that treatment with sulphasalazine, but not other disease-modifying antirheumatic drugs, resulted in a decline in anti-CCP antibody titers, but this decrease only occurred in the first year of the follow-up. Applications of disease-modifying antirheumatic drugs have been reported to result in a small reduction in anti-CCP antibody in about 50% of patients (Mikuls et al. 2004). Alessandri et al. (2004) have also found a small but significant decrease in anti-CCP antibody levels in the patients showing clinical improvement and being treated with infliximab/methotrexate, but not in those treated with methotrexate alone. Thus not to evaluate the effects of DMARDs usage on anti-CCP antibody titer may be another limitation of our study.

In conclusion, results from this study indicate that the anti-CCP antibody is more specific and exhibits better diagnostic value than IgM-RF for RA, and it may be more predictive for radiological progression of RA. Consequently, anti-CCP antibody may be useful to anticipate the prognostic process in RA and to make a decision for the intensity of treatment regimens. Importantly, the results also suggest that anti-CCP antibody is not associated with BD.

References
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