Prevalence of anti cyclic citrullinated peptide antibodies in Malaysian rheumatoid arthritis patients and its correlation with disease activity

Sargunan Sockalingam,1 Chow Sook Khuang2 and Pavai Sthaneshwar3

1Department of Internal Medicine, Faculty of Medicine, University Malaya, 2Sunway Medical Centre, Visiting Consultant, University Malaya Medical Centre and 3Chemical Pathology Unit, Faculty of Medicine, University Malaya, Kuala Lumpur, Malaysia

Abstract

Aim: The objectives of this study are to provide data regarding the prevalence of anti-cyclic citrullinated peptide (CCP) antibodies in Malaysian rheumatoid arthritis (RA) patients and to correlate the levels of anti-CCP antibody with the Disease Activity Score (DAS).

Method: We studied the prevalence of anti-CCP antibodies in 51 RA patients attending our clinic and 29 controls. We also looked for correlation between anti-CCP antibody levels with the DAS and parameters such as duration of disease, rheumatoid factor (RF) and disease-modifying anti rheumatic drug (DMARD) usage.

Results: None of the controls demonstrated anti-CCP antibodies. Forty-one out of 51 patients (80.4%) were positive for anti-CCP antibodies. Sensitivity and specificity were 80.4% and 100% respectively in this study. Anti-CCP levels correlated significantly with rheumatoid factor, but no correlation was observed with the other parameters.

Conclusions: Anti-CCP antibody is prevalent in Malaysian RA patients at 80.4% and more sensitive than RF in our cohort of established RA patients. Even though the anti-CCP levels correlated with RF, it did not show correlation with DAS.

Key words: anti-cyclic citrullinated peptide antibodies, clinical aspects, DAS 28, rheumatoid arthritis, rheumatoid factor.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disorder of unknown etiology characterized by symmetric and erosive synovitis. It causes progressive joint destruction and disability. In the United States alone, RA results in more than 9 million physician visits and more than 250 000 hospitalizations per year.1 Early diagnosis and the ability to predict who will develop erosive disease are important as these patients can be treated early and deformities can be prevented.

Correspondence: Dr Sargunan Sockalingam, Department of Medicine, Faculty of Medicine, University Malaya, 50603 Kuala Lumpur, Malaysia. Email: sargunan@um.edu.my

Rheumatoid factor (RF) was identified in RA patients over 50 years ago. However, RF is also found in 5% of healthy persons and its prevalence in the general population increases with age. RF will be positive in 10–20% of individuals over the age of 65. In addition, a number of conditions besides RA, such as connective tissue diseases and infections, are associated with positive RF. RF may appear transiently in normal individuals following vaccination or transfusion and may also be found in relatives of individuals with RA.2,3 The RF assay, in its current form remains suboptimal as a diagnostic test. It lacks sensitivity (54–88%) and specificity (48–92%).4–6 However, it has been established that high titers of RF indicate aggressive disease.1–3
The shortcomings of the RF assay have provided impetus for identification of other serological assays for RA. Autoantigens such as antikeratin antibodies (AKA), Sa, BiP, RA33, glucose-6-phosphate isomerase, and anti-perinuclear factor (APF or anti-fillagrin) are present almost exclusively in patients with RA. Analysis of AKA and APF auto-antibodies showed that most of the reactivity present against these antigens was directed against citrulline residue, a post-translational modification of the amino acid arginine. This discovery led to the development of assays employing cyclic citrullinated peptides (CCP) to measure antibodies recognizing citrullinated antigens as a diagnostic test for RA. A positive value for anti-CCP predicts the future development of erosive RA and also predicts the development of more severe disease outcome.

The purpose of this study is to know the prevalence of anti-CCP antibodies in Malaysian RA patients. We also looked at correlation between anti-CCP antibodies with RF, the Disease Activity Score (DAS), duration of disease and disease-modifying anti-rheumatic drug (DMARD) usage.

MATERIALS AND METHODS

This was a cross-sectional, hospital-based study of patients with established RA attending the Rheumatology Clinic at the University Malaya Medical Centre (UMMC) within a 2-month period from February 2004 to March 2004. Ethics Committee of the University Malaya Medical Center approval was obtained prior to the initiation of the study. Every effort was made to conform to the provisions of the World Medical Association’s Declaration of Helsinki.

A total of 51 RA patients were consecutively selected from our clinics. Patients fulfilled the American College of Rheumatology (ACR) diagnostic criteria for RA. Informed verbal consent was obtained. Data on age, gender, ethnic group, duration of RA disease and number/type of DMARDs used were collected via patient interview using a questionnaire. The numbers of swollen, tender and damaged joints were noted by the physician. Joints which were fixed and/or subluxated were considered as damaged. Details regarding extra-articular manifestations and arthroplasty were also noted. Blood samples for erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), RF and anti-CCP antibody assays were collected.

The control group comprised of healthy hospital workers of UMMC, between the ages of 40–60 years who did not suffer from any known rheumatic diseases. They were matched with the cases for age, sex and ethnicity.

Disease activity was assessed using the visual analogue score. Activity was scored from 1 to 100 mm on three separate scales. The first and the second visual analogue scores were done by the patient who reports on the wellbeing and the global assessment of RA activity respectively. The third scale was the physician’s global assessment of RA activity.

The Rheumatoid Arthritis Disease Activity Score (DAS28) developed by Prevoo et al. was used to define disease activity in our patients. This scoring system is based on the number of tender and swollen joints (n = 28), ESR, and the patient’s self-estimated level of disease activity. This score may range from 0 to 9.3, where a DAS28 score ≤ 3.2 is considered to reflect low disease activity and a DAS28 score > 5.1 indicates high disease activity.

Anti-CCP antibody was measured using the QUANTA Lite (Inova Diagnostics Inc., San Diego, CA, US) CCP IgG enzyme-linked immunosorbent assay (ELISA). It is a semi-quantitative enzyme linked immunosorbent assay for the detection of IgG anti-CCP IgG antibodies.

The data were analyzed using SPSS version 11.5 statistical software package (SPSS Inc, Chicago, IL, US). A breakdown descriptive statistical analysis was carried out and a P-value of < 0.05 was considered statistically significant. As the number of patients studied was small, and the distribution skewed, the median was used instead of the mean for comparison. Chi-squared tests were used for the comparison of anti-CCP with the disease parameters. Non-parametric method was used to compare medians. Correlation data was obtained using linear regression and binary logistics models.

RESULTS

Demographic data of patients and controls are represented in Tables 1 and 2 and represent the median values of the various parameters of disease activity among patients and percentage values of the other clinical parameters of interest. Both clinical and laboratory parameters were assessed. The DAS28 had a median value of 4.11 with a range of 0.28–8.30.

The reference range of anti-CCP antibody levels obtained for our study population was 0.3–13.2 units. Anti-CCP antibody level of above 20 units was considered as positive.
Table 1 Demographic data of the studied controls (n = 29) and patients (n = 51)

<table>
<thead>
<tr>
<th>Demographic parameter</th>
<th>Controls</th>
<th>Patients</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range)</td>
<td>51 (34–62)</td>
<td>51 (23–80)</td>
<td>0.912</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>0.0%</td>
<td>5.9%</td>
<td>0.188</td>
</tr>
<tr>
<td>Females</td>
<td>100%</td>
<td>94.1%</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td>0.157</td>
</tr>
<tr>
<td>Malay</td>
<td>28%</td>
<td>27%</td>
<td></td>
</tr>
<tr>
<td>Chinese</td>
<td>58%</td>
<td>40%</td>
<td></td>
</tr>
<tr>
<td>Indian</td>
<td>14%</td>
<td>25%</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>0%</td>
<td>8%</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Median values and range of the various parameters used in assessment of rheumatoid arthritis disease activity and percentage values in disease severity

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No. patients</th>
<th>Median (range) percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease activity Clinical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tender joints</td>
<td>1 (0–25)</td>
<td></td>
</tr>
<tr>
<td>Swollen joints</td>
<td>3 (0–24)</td>
<td></td>
</tr>
<tr>
<td>Patients global RA activity</td>
<td>48 (2–95)</td>
<td></td>
</tr>
<tr>
<td>Physician global RA activity</td>
<td>44 (4–95)</td>
<td></td>
</tr>
<tr>
<td>Disease activity score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESR</td>
<td>41 mm/h (1–135)</td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>0.68 mg/dL (0–18.9)</td>
<td></td>
</tr>
<tr>
<td>Disease severity Clinical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Damaged joints</td>
<td>0 (0–38)</td>
<td></td>
</tr>
<tr>
<td>Extra articular manifestations</td>
<td>9</td>
<td>17.6%</td>
</tr>
<tr>
<td>1 DMARD</td>
<td>28</td>
<td>54%</td>
</tr>
<tr>
<td>2 DMARDs</td>
<td>17</td>
<td>33%</td>
</tr>
<tr>
<td>&gt; 2 DMARDs</td>
<td>3</td>
<td>5.9%</td>
</tr>
<tr>
<td>0 DMARDs</td>
<td>3</td>
<td>5.9%</td>
</tr>
<tr>
<td>Arthroplasty</td>
<td>4</td>
<td>7.8%</td>
</tr>
<tr>
<td>Laboratory Rheumatoid factor</td>
<td>17 IU/mL (0–591)</td>
<td></td>
</tr>
</tbody>
</table>

RA, rheumatoid arthritis; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; DMARD, disease-modifying antirheumatic drugs.

Anti-CCP levels in controls were < 13.2 units and none had values higher than this. The median anti-CCP antibody level in the control population was 1.2 units (range from −2.5 to 11.4). RF was noted to be positive in five of the controls.

Out of 51 cases of RA, 41 had elevated levels of anti-CCP antibodies and the remainder had the levels within the reference range. The median anti-CCP antibody level was 194.6 (−0.7 to 332.7).

The sensitivity and specificity of anti-CCP were 80.4% and 100% respectively in our study cohort group. RF showed lesser sensitivity and specificity (72.5% and 82.7%) compared to anti-CCP.

Anti-CCP antibody levels did not correlate with the number of tender joints (P = 0.478), swollen joints (P = 0.417), patients’ global assessment of disease activity (P = 0.562) or physician assessment of RA activity (P = 0.282). No correlation was observed between anti-CCP antibody levels and either ESR or CRP (P > 0.05). We also compared the DAS, the duration of RA and the numbers of DMARDs used between the anti-CCP positive group and the anti-CCP negative group of patients. There was no significant difference in any of the above parameters between anti-CCP positive groups and anti-CCP negative groups (P > 0.05). RF showed a positive Pearson correlation (r = 0.582) with anti-CCP antibody titre (P < 0.05).

DISCUSSION

In this cross-sectional observational study we tested a new serological assay that might help us improve diagnosis and management of RA.

Our study showed a prevalence of anti-CCP antibody level of 80.4% in the disease population. Our study included only established RA patients, while other studies compared a wider cohort of patients, such as palindromic rheumatism, undifferentiated arthritis and other connective tissue diseases.9,10,15,16

None of the controls had elevated levels of anti-CCP antibody. In a study by Low et al.,17 they found no elevated anti-CCP antibody levels among their 25 healthy controls. However, our control population did not include people with other connective tissue disorders such as systemic lupus erythematosus (SLE), psoriatic arthropathy, systemic sclerosis, and so on. In this study, the anti-CCP antibody was more specific than RF which was positive in five (17.2%) of the controls. In our cohort of RA patients the anti-CCP antibody assay was also more sensitive than the RF (80.4% vs. 72.5%). In a study by Wu et al.,18 sensitivity of anti-CCP was 84%, compared to RF (78%) in patients with refractory RA. However, other studies
have reported a lower sensitivity of anti-CCP when compared to RF.\(^{16,19}\)

Comparison between the anti-CCP antibody positive and negative groups did not show any significant difference in the DAS. This is in agreement with Kastbom et al.\(^{20}\) where they found no difference in the DAS between these two groups at baseline in their cohorts of early RA patients.

It is well known that RF positivity is associated with more aggressive and erosive disease.\(^{1–3}\) In this study, there was a moderate positive correlation between anti-CCP antibody and RF (\(r = 0.582, P < 0.05\)). Studies have also reported a correlation between anti-CCP antibody and radiologically evident erosive disease.\(^4\)

The limitation in our study was that we failed to collect information about history of smoking. Smoking has been known to be a risk factor for the development of seropositive RA.\(^{21,22}\) Citrullinated peptides have been detected in the broncho-alveolar lavage in RA patients who smoke.\(^{23}\) Klareskog et al.\(^{24}\) found that smoking was associated with the presence of anti-CCP antibodies in RA patients.

The shared epitope HLA-DRB1 has been found to be strongly associated with the production of anti-CCP antibodies.\(^{25}\) Since HLA typing is not routinely performed in RA patients, we did not study the correlation of HLA-DRB1 with anti-CCP.

In conclusion, anti-CCP antibody is prevalent in Malaysian RA patients. Therefore anti-CCP antibody assay is a test that is applicable to the Asian population. As none of the controls showed a positive anti-CCP assay, its role in the diagnosis of RA is comparable with, if not better than, RF. There is a significant correlation between anti-CCP antibody titer and RF levels, therefore making it a potential prognostic indicator. The anti-CCP antibody assays have no significant correlation with the DAS, disease duration and DMARD usage. With this background of anti-CCP antibody assay as a diagnostic tool and its correlation with RF, further studies can be conducted in our centre to analyze its specificity when studied along with other rheumatic diseases. There is also significant scope for further correlation studies with disease severity using the modified Health Assessment Questionnaire (HAQ) and measures of radiologically evident erosions, such as the Sharp-van der Heijde score.

**ACKNOWLEDGMENTS**

This study was funded by the Rheumatology Research Fund, University Malaya. We wish to acknowledge the staff of the Rheumatology Clinic, University Malaya Medical Center (UMMMC) and the Clinical Biochemistry Laboratory, UMMC for assistance in the collection of data and blood samples. We also would like to thank Mr Darssan Balasingam, Biostatistician at the St George Hospital, Sydney for his assistance in the statistical analysis of the acquired data.

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