Evaluation of Anti-CCP Antibody for Diagnosis of Rheumatoid Arthritis

NADEEM AFZAL 1, SARA KARIM 1, TAFAZZUL-E-HAQE MAHMUD 2, WAQAS SAMI 1, MARIA ARIF 1, SARWAR ABBAS 1

1 Department of Immunology, University of Health Sciences Lahore, Pakistan
2 Department of Rheumatology & Immunology Sheikh Zayed Hospital, Lahore, Pakistan

SUMMARY

Background: Rheumatoid arthritis is a common, world wide, systemic disease that affects mainly joints. Rheumatoid factor is the only marker to diagnose rheumatoid arthritis; however these antibodies are present in other disorders and even in up to 15 % of the healthy population. Many auto antibodies have been reported to diagnose rheumatoid arthritis e.g. APF and AKA, etc. but they are not specific and due to tedious laboratory procedure, they have not been generally adopted. Anti-CCP antibodies have been reported for their high sensitivity and specificity. This study was planned to determine the prevalence of anti-CCP antibodies and RA factor in clinically diagnosed patients of rheumatoid arthritis.

Methods: Anti-CCP antibody was determined by ELISA technique and RA-factor was done by latex agglutination method.

Results: Forty five patients, 36 female and 9 male, were recruited for this study. Twenty-five (55.6 %) patients were positive for anti-CCP antibodies while 20 patients were negative for anti-CCP antibodies and comparison between anti-CCP positive and anti-CCP negative was statistically significant (p = <0.01). Thirty-one (68 %) patients were seropositive (SPRA) for RA while 14 (31 %) patients were seronegative (SNRA). Among SPRA patients, 18 were positive for anti-CCP antibody and among 14 SNRA patients, 7 patients had anti-CCP antibody and the difference between these two groups was not statistically significant.

Conclusions: Anti-CCP antibody and RA-factor should be used concomitantly to diagnose RA.

(Clin. Lab. 2011;57:895-899)

KEY WORDS

Rheumatoid factor, anti-perinuclear factor, anti keratin antibody, anti-cyclic citrullinated peptide antibody

LIST OF ABBREVIATIONS

RA: Rheumatoid arthritis
ACR: American College of Rheumatology
Anti-CCP: anti-cyclic citrullinated peptide
SPRA: Seropositive
SNRA: Seronegative
ELISA: Enzyme linked immunosorbant assay
APF: Anti-perinuclear factor
AKA: Anti-keratin antibodies
ANCA: Anti-neutrophil cytoplasmic antibody

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease of unknown etiology associated with chronic inflammation of joints and production of several auto antibodies that often lead to joint destruction and disability [1,2]. Its diagnosis requires at least four of the criteria mentioned in the list of American College of Rheumatology (ACR). Its prevalence is about 1 % world wide and is more common in women than men (2.5:1) [3].
Rheumatoid arthritis is a complex disease where several genetic, environmental, and stochastic factors are responsible for its development. Genetic susceptibility is significant for its development as its prevalence is high in monozygotic twins [4]. Studies showed association between shared epitope of HLA-DRβ allele with RA [5] and HLA-DRB1 with severity of disease. [6,7] HLADRB1 01 and HLADRB1 03 were found more common in Pakistani RA patients. [8] Rheumatoid arthritis is associated with several auto antibodies i.e. rheumatoid factor (RF), anti-perinuclear factor (APF), ANCA, anti-RA33, anti-flaggerin antibodies, anti-keratin antibodies (AKA), anti-cyclic citrullinated peptide antibodies (CCP), etc. [9]. APF and AKA antibodies showed high specificity but due to technical difficulties and tedious laboratory procedures they were not generally adopted [10,11]. APF and AKA antibodies also targets citrullinated proteins. [12] Rheumatoid factor (RF) can be detected in 60 % - 80 % of RA patients and it is also found in other connective tissue diseases, chronic inflammatory diseases, and various infections such as malaria, in the elderly population and even in healthy individuals [13,14]. Although RF is not a specific test for RA, the determination of RF is included in the ACR criteria to diagnose RA.

Citrullination is a post-translational modification of protein-bound arginine into the non-standard amino acid citrulline. Enzymatic conversion of arginine to citrulline is catalyzed by the peptidylarginine enzyme [2,15]. Anti-CCP antibodies can react with several citrullinated peptides on multiple proteins such as flaggerin, vimentin, fibrin, and alpha enolase. Therefore, these antibodies are labeled as anti-citrullinated protein/peptide antibodies (ACPA). Among the various auto antibodies, RF and anti-CCP antibodies are considered useful diagnostic markers for RA [2].

Anti-CCP antibodies were first described by Schellekens et al for their high specificity (98 %) and high sensitivity (79 %) in the diagnosis RA [16]. Scientists reported their specificity between 95 % - 98 % and suggested their increased specificity as an advantage [17]. Anti-CCP antibodies have been detected in the sera of patients even before the appearance of characteristic signs and symptoms of RA i.e. in the pre-clinical stage of disease [15,18]. Anti-CCP antibodies were detected at an early stage of seronegative RA patients [19], therefore, these antibodies were suggested as a useful diagnostic tool, particularly in early stages of RA [20]. First generation anti-CCP test kits had the sensitivity of 60 % - 68 % while 2nd generation had a sensitivity of 75 % - 80 % and currently 3rd generation anti-CCP antibody detection kits greater sensitivity are being used [21].

In juvenile idiopathic arthritis (JIA), anti-CCP antibody was associated with RF positive polyarticular patients and, therefore, it was suggested that anti-CCP should not be investigated routinely in JIA patients [22]. Later, presence of these antibodies in both IgM-RF-positive and IgM-RF-negative JIA children even at an early stage of the disease was documented [23].

Connective tissue diseases such as systemic lupus erythematosus (SLE), Sjogren’s syndrome (SS), etc. can manifest with joint involvement which make these diseases indistinguishable from RA. In a retrospective study, anti-CCP distinguished RA, SLE, and other deforming arthropathies and, therefore, scientists suggested that anti-CCP antibody was linked more to RA than SLE [24]. Low prevalence of anti-CCP in isolated SLE patients confirmed the observation that anti-CCP was almost completely confined to RA. Sjogren’s syndrome also shares several clinical features with RA but anti-CCP antibodies were found in only 7.5 % of patients with primary Sjogren's syndrome as compared to patients of RA [25]. Another auto antibody i.e. anti-MCV, has been reported which is directed against citrullinated vimentin and is reactive with citrullinated proteins. However, sensitivity and specificity of anti-MCV antibody was 69.5 % and 90.8 %, respectively, compared to 70.1 % and 98.7 % for the anti-CCP 2 assay. Therefore, due to high specificity, determination of anti-CCP is considered a test to superior anti-MCV for the diagnosis of RA [26]. Although sensitivity of RF and anti-CCP antibody was the same, anti-CCP proved a better predictor of the course of disease over a period of 3 years [27] and concentration of anti-CCP antibodies increased with the severity of disease [28]. Patients with anti-CCP antibodies developed more severe disease with more radiological destruction compared to RA patients who did not have these auto antibodies [29].

Considering the high specificity and sensitivity of anti-CCP antibody for RA, it was decided to carry out a study to determine the frequency of anti-CCP antibody and RA factor in clinically diagnosed RA patients. The aim was to find out a dependable marker for the diagnosis of RA because a reliable serological marker for RA which should be sensitive, highly specific with the ability to detect the disease at early stage and should have prognostic value as well, is urgently needed.

**MATERIALS AND METHODS**

Forty five (45) clinically diagnosed RA patients between 20 and 60 years from both genders were selected from the Department of Rheumatology and Immunology, Sheikh Zayed Postgraduate Institute, Lahore. A revised ACR criteria for RA has been developed in collaboration with the EULAR but for this study patients were recruited according to the ‘1987 criteria for the classification of acute arthritis of RA’ [30] i.e. morning stiffness, minimum three joint involvement, at least one swollen joint, symmetric involvement, enlargement of subcutaneous nodules, positive rheumatoid factor, and radiologic changes. These patients were divided into two groups; Group 1 included 31 (68.8 %) rheumatoid factor positive (Sero-positive-RA: SPRA) and group 2 included 14 (31.1 %) rheumatoid factor negative (Sero-negative-RA: SNRA) patients. On the basis of morning
Table 1. Percentages and mean ± SD of anti-CCP antibodies.

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percentage %</th>
<th>Mean ± SD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-CCP positive</td>
<td>25</td>
<td>55.6</td>
<td>2315.86 ± 1967.231</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Anti-CCP negative</td>
<td>20</td>
<td>44.4</td>
<td>25.00 ± .000</td>
<td></td>
</tr>
</tbody>
</table>

*p value <0.05: statistically significant

Table 2. Rheumatoid factor and anti-CCP antibodies.

<table>
<thead>
<tr>
<th>RF Status</th>
<th>Anti-CCP Positive N (%)</th>
<th>Anti-CCP Negative N (%)</th>
<th>Anti-CCP Mean ± S.D</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPRA (31)</td>
<td>18 (40.0 %)</td>
<td>13 (28.9 %)</td>
<td>1574.96 ± 2080.748</td>
<td>0.137</td>
</tr>
<tr>
<td>SNRA (14)</td>
<td>7 (15.6 %)</td>
<td>7 (15.6 %)</td>
<td>1024.543 ± 273.821</td>
<td></td>
</tr>
</tbody>
</table>

N = number of patients, % of anti-CCP antibodies
*p value <0.05: statistically significant

Table 3. Anti-CCP antibody levels in patients with morning stiffness.

<table>
<thead>
<tr>
<th>Morning stiffness</th>
<th>Anti-CCP positive</th>
<th>Anti-CCP negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild (n = 13) (28.89 %)</td>
<td>8 (17.8 %)</td>
<td>5 (11.1 %)</td>
</tr>
<tr>
<td>Moderate (n = 17) (37.78 %)</td>
<td>10 (22.2 %)</td>
<td>7 (15.6 %)</td>
</tr>
<tr>
<td>Severe (n = 10) (22.22 %)</td>
<td>5 (11.1 %)</td>
<td>5 (11.1 %)</td>
</tr>
<tr>
<td>Total (n = 40)</td>
<td>23</td>
<td>17</td>
</tr>
</tbody>
</table>

n = number of patients

Statistical Analysis

The data was entered and analyzed using SPSS 18.0. Mean ± S.D was given for quantitative variables. Frequencies and percentages were given for qualitative variables. Two independent sample t tests were applied to observe mean difference between positive and negative Anti-CCP antibodies. A p-value of <0.05 was considered statistically significant.

RESULTS

Forty five patients comprising 36 (80 %) females and 9 (20 %) males were recruited for this study. The ratio of male to female was 4:1 and mean age ± SD of patients was 36.80 ± 13.73. Twenty-five (55.6 %) patients were positive for anti-CCP antibodies while 20 patients were negative for
anti-CCP antibodies. Mean ± SD of anti-CCP antibody level was 1297.70 ± 1853.69 and the comparison between two groups i.e. anti-CCP positive and anti-CCP negative was statistically significant (p < 0.01). (Table 1) On performing RF, 31 (68 %) patients were seropositive for RA (SPRA) while 14 (31 %) patients were seronegative (SNRA). Among SPRA patients, 18 (58 %) patients were positive for anti-CCP antibody and among 14 SNRA patients, 7 (50 %) patients had anti-CCP antibody (Table 2). The difference between these two groups was not statistically significant (p = 0.137).

Regarding morning stiffness 13 (28.9 %) patients had mild, 17 (37.8 %) had moderate and 10 (22.2 %) had severe complaints while 5 patients had no such complaints. The number of patients and percentages in each group of positive or negative anti-CCP antibodies is given in Table 3.

In our study population, 12 (26.7 %) patients presented with the complaints of mild fatigue, 21 (46.7 %) with moderate and 11 (24.4 %) had severe complaints of fatigue whereas 1 patient had no complaint. Among patients with mild complaint, 5 (11.1 %) had anti-CCP antibodies and from patients with moderate complaint, 11 (24.4 %) had anti-CCP antibodies whereas among patients with severe complaint of fatigue, 8 (17.8 %) had anti-CCP antibodies.

In the study population, 18 (40 %) patients had a family history of RA and among them 13 were SPRA while 27 (60 %) patients did not have a family history of RA and among them, 18 patients were SPRA.

**DISCUSSION**

The present study was carried out to determine the frequency of anti-CCP antibodies and RF in clinically diagnosed RA patients. An attempt was also made to determine the number of patients who had anti-CCP antibodies among SPRA and SNRA patients.

In the SPRA group, 18 (40 %) patients had anti-CCP antibodies while 13 (30 %) did not have anti-CCP antibodies, whereas in the SNRA group, 7 (15.6 %) patients were positive for anti-CCP antibodies. Our findings are in agreement with Kastbom et al [27] who also showed anti-CCP antibodies in SNRA patients with 60 % sensitivity and 92 % specificity. Therefore, he suggested that anti-CCP antibodies could be found before a patient becomes seropositive for RA. The difference in the level of specificity and sensitivity with our study could be due to the use of third generation anti-CCP kits. Likewise, Nicola et al showed anti-CCP antibodies in healthy blood donors before the appearance of typical clinical signs and symptoms of RA [28].

In our study, among 31 SPRA patients, 13 patients were negative for anti-CCP antibodies. It suggested the possibility that these patients might not be actually suffering from RA. It also favored the statement that RF might not be a specific marker for the diagnosis of RA and it could be present in other connective tissue disorders. Further, these patients could be in a quiescent stage of disease, therefore anti-CCP antibodies could not be detected or titer of these antibodies was less than 25 IU/L. We found that levels of anti-CCP antibodies were high in patients who were placed in the moderate group of pain intensity as compared to patients placed under mild and severe group of pain. Yazici et al. also confirmed that morning stiffness in early stages of RA was associated with more functional disability [31]. In our study, among 31 SPRA patients 13 were negative for anti-CCP antibody and all of these patients were suffering from RA for the last 10-12 years. This result is similar to some extent with the findings of Kastbom et al who did a study on 242 RA patients and followed them for 3 years. He concluded that anti-CCP antibody is a good predictor of the course of disease, because 2 patients who were negative for anti-CCP antibodies became positive for this antibody while 3 patients who were positive for anti-CCP antibodies became negative for this test during the study period. In our studied population there were 18 (40 %) patients with a family history of RA and they were positive for anti-CCP antibodies which supported the familial predisposition of RA [4].

**CONCLUSION**

We found anti-CCP antibody a slightly better marker for the diagnosis of RA and anti-CCP antibody also assisted in diagnosing patients who did not have a family history of RA. However, RA-factor is still a reliable marker. It is suggested that both of these markers should be used concomitantly to diagnose RA to benefit the sufferers.

**Disclaimers:**
The study was carried out as a research project of a M. Sc student.

**Support in the form of grant and equipment:**
University of Health Sciences, Lahore, Pakistan.

**Declaration of Interest:**
None of the authors have any interest in the kits/products used in the study.
COMPARISON OF ANTI-CCP ANTIBODIES AND RHEUMATOID FACTOR FOR THE DIAGNOSIS OF RHEUMATOID ARTHRITIS

References:


5. De Vries RRP, Huizinga TWJ, Toes REM. Redefining the HLA and RA association: To be or not to be-anti-CCP positive. J Autoimm 2005;25:21-5.


Correspondence:
Nadeem Afzal Assistant Professor and Senior Consultant Head
Department of Immunology
University of Health Sciences
Lahore, Pakistan
Tel.: +92-42-99231304-9 Ext 343
E-mail: immunology@uhs.edu.pk

Clin. Lab. 11+12/2011 899