Anti-CCP Antibodies in Unaffected First-Degree Relatives of Rheumatoid Arthritis Patient

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Abstract

Introduction: Rheumatoid arthritis (RA) is perhaps the most common inflammatory arthritis, affecting 0.5% to 1% of the general population worldwide. Environmental and genetic influences are important risk factors for pathogenesis of RA. First-degree relatives (FDRs) of RA patients sharing genetic and environmental risk factors for RA may represent as pre-RA state. Since anti-CCP antibody appears years before the onset of RA, it can be used to estimate risk-potential for future development of RA in unaffected FDRs of RA patient. Objective: To see the serum anti-CCP antibody in unaffected FDRs of RA patients. Materials and Methods: This cross-sectional study was conducted from July 2019 to June 2020 in Department of Biochemistry, Dhaka Medical College, Dhaka. A total number of 50 subjects were selected among which 25 were unaffected FDRs of RA patients and the other 25 were age & gender matched healthy subjects. The related RA patients were selected from Rheumatology outdoor, Department of Rheumatology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka and the healthy subjects were taken from the attendants came to the same institute who did not have RA patient in their family. The key variables (age and gender) were recorded and outcome variable (anti-CCP antibody in study subjects) was estimated. Statistical analysis was done by SPSS 26.0 and the significance was defined as p < 0.05. Results: Mean age of the FDRs of RA patient was 31.36 ± 10.51 years and that of healthy subjects was 31.56 ± 8.89 years. Most of the participants were in age group 20-29 years followed by 30-39 years. 4% of FDRs of RA patient were anti-CCP antibody positive and no positive case was recorded in healthy subjects. The median level of anti-CCP antibody in FDRs of RA patient was 0.50 U/mL and that in healthy subjects was 0.09 U/mL. The level of significance (p = 0.002) showed that serum anti-CCP antibody level was significantly higher (within normal range) in FDRs of RA patient than in healthy subjects, which was even more significant in FDRs of seropositive RA patients. Conclusion: This study revealed that serum anti-CCP antibody level was significantly higher (within normal range) in FDRs of RA patient than in healthy subjects and the proportion of positive anti-CCP antibody was more in FDRs of RA patient than in healthy subjects.

Key words: Anti-CCP antibodies, rheumatoid arthritis, first-degree relatives.

Number of Tables: 06; Number of Figures: 02; Number of References: 28; Number of Correspondences: 03.
Introduction:
Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by joint swelling, joint tenderness and destruction of synovial joints, leading to severe disability and premature mortality. It is perhaps the most common inflammatory arthritis, affecting 0.5% to 1% of the general population worldwide and predominantly affects females (2-3 fold more often than males). RA can occur at any age but it is more frequent among individuals in the fourth to sixth decades of life. If untreated, 20%-30% of RA patients become so severely debilitated within the first three years following initial diagnosis that they become permanently disabled.

The precise causes of RA remain uncertain but environmental and genetic influences clearly are factors. Smoking exposure has a dose response relationship with RA risk. Genetic studies have demonstrated that a genetic predisposition resides in the human leukocyte antigen (HLA)-DR locus. In monozygotic twins there is about 15% to 30% concordance rate for rheumatoid arthritis development.

RA is primarily considered as a disease of the joints, but multiple additional organ systems are also known to be involved including pulmonary, cardiovascular, ocular and cutaneous systems.

The initiation of RA begins years before the onset of clinical symptoms. The onset involves certain specific genes that can lead to the production of pathogenic antibodies that bind to modified proteins, help break tolerance, and lead to autoreactivity.

The standard and accepted means of defining RA is by use of classification criteria. This classification was essentially based on the criteria formulated by the American College of Rheumatology (ACR; formerly the American Rheumatism Association) in 1987. These criteria exhibit 91%–94% sensitivity and 89% specificity for established RA, but have a significant limitation and are therefore not helpful in achieving the goal of identifying patients who would benefit from early effective intervention. A systematic review of publications evaluating the performance of the ACR’s 1987 clinical criteria found the sensitivity to early RA to be between 77% and 80%, with specificities between 33% and 77% based on pooled data. To overcome this limitation a joint working group of the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) was formed and they developed the 2010 ACR/EULAR classification criteria for RA.

To meet the need for improved diagnostic and prognostic tests and algorithms, various serum biomarkers are being assessed including a wide range of autoantibodies. However, only rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) have gained wide acceptance.

The first autoantibody in RA, rheumatoid factor (RF), was described by Waaler in 1940, and it was later found to be directed to the Fc region of IgG. IgM RFs are the major RF species in RA and are found in approximately 60%-80% of these patients. It is also found in patients with infections, other autoimmune diseases, and some unaffected individuals with increasing frequency in older age groups, thus limiting its specificity for RA. It is found in 3-5% of the general population, and in up to 30% of the elderly. Historically, RF was the main element in the serological diagnosis of RA and the only laboratory diagnostic parameter included in the 1987 American College of Rheumatology (ACR) criteria for the classification of RA.

Early diagnosis and treatment in RA are crucial as it can prevent disease progression and irreversible joint damage but RF lacks RA specificity and has a low prevalence in new-onset disease. So, anti-citrullinated protein antibodies (ACPAs) have been included in the 2010 ACR/EULAR classification criteria for RA as the second serological marker, which are focused on early diagnosis and therapy. ACPAs are locally produced in RA joints, where proteins are citrullinated during the inflammatory process, which are found in 70-90% of RA patients and have high disease specificity (90-95%). Accordingly, they are rarely found in other diseases or in unaffected individuals.

The more recent autoantibodies for the diagnosis of RA are anti-cyclic citrullinated peptide antibodies (anti-CCP antibodies). Anti-CCP antibody testing is particularly useful in the diagnosis of RA and it is able to predict the severity of the disease and the irreversible damage. It has been shown to be reasonably sensitive (65-80%) and highly specific for RA (up to 98%) due to high specificity and moderate sensitivity, a positive anti-CCP antibody result means RA is likely though a negative result does not rule out the disease.

There is evidence that the presence of anti-CCP antibodies can be detected long (up to 15 years) before the onset of clinical manifestations which suggests their role in the pathogenesis of RA.

The first ACPAs in RA sera were discovered by Nienhuis et al. in 1964, and were named antiperinuclear factor (APF). In 1979, Young et al. reported that RA sera contained antikeratin antibodies (AKA). These antibodies are highly specific for RA (up to 99%), but unfortunately, the inconvenient test format and inconvenient antigenic substrates used to determine the presence of the APF/AKA antibodies have hindered their use as a serological marker.

In 1998, Schelleken reported that antibodies, against citrullinated peptide, are highly specific seromarker for the diagnosis as well as prognosis of rheumatoid arthritis. Schellekens et al. designed a synthetic cyclic citrullinated peptide (CCP) and used it as a new antigenic substrate in anti-CCP ELISA (Enzyme Linked Immunosorbent Assay) to detect ACPAs. The first generation of anti-CCP antibodies (anti-CCP1) test revealed a higher specificity for RA in comparison to the RF test. At the end of 2002, second generation anti-CCP (anti-CCP2) antibodies tests were developed, with different cyclic peptides and improved performance characteristics showing an even better specificity for RA.

Several studies have examined the performance characteristics of anti-CCP antibodies in RA, using both the anti-CCP1 and
anti-CCP2 assays. Sensitivity and specificity using the anti-CCP1 assay ranged from 44% to 56% and 90% to 97%, respectively. Detection of antibody with CCP2 assays resulted in improved sensitivity (64–89%), and specificity (88–99%). Rheumatoid factor sensitivity ranged from 59% to 79% and specificity from 80% to 84% in the same groups.

Nishimura et al. performed a meta-analysis of published studies regarding the diagnostic accuracy of anti-CCP and RF for rheumatoid arthritis. Their results showed a positive likelihood ratio of 12.46 and a negative likelihood ratio of 0.36 for anti-CCP antibody in patients with RA. The same study showed a positive likelihood ratio of 4.86 and a negative likelihood ratio of 0.38 for RF. These results indicate that anti-CCP positivity alone is more specific than IgM RF for the diagnosis of RA.

Studies on anti-CCP antibodies in unaffected first-degree relatives of RA patients are very few in Bangladesh. So, the aim of this study was to see the serum anti-CCP antibody in unaffected first-degree relatives of RA patients and compare with those of non-related healthy subjects.

Materials and Methods:
This cross-sectional study was conducted in the Department of Biochemistry, Dhaka Medical College, Dhaka during the period of July 2019 to June 2020. For this study, a total number of 50 subjects between 20-60 years of age were selected, among which 25 were unaffected first-degree relatives (FDRs) of rheumatoid arthritis (RA) patients and the other 25 were age & gender matched healthy subjects. The related RA patients were selected from Rheumatology outdoor, Department of Rheumatology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, and the healthy subjects were taken from the attendants came to the same institute who did not have RA patient in their family. Informed written consent was taken from the study subjects after full explanation of procedure regarding study in a prescribed form. A prefixed questionnaire was used to record detailed history, relevant physical examinations and investigations. After all aseptic precaution 5 ml of venous random blood sample was collected from each study subject.

All the biochemical tests were performed in the department of Biochemistry, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, estimated by Enzyme-linked Immunosorbent Assay (ELISA) method. All data were recorded in a predesigned data collection sheet. Data was analyzed by using the statistical package for social sciences (SPSS) software version 26.0. Descriptive analysis was performed. Continuous variables were expressed as mean ± SD or median (IQR) and were compared between groups of subjects by unpaired student’s t-test or Mann-Whitney U test. Categorical variables were compared using a Chi-square test or Fischer’s exact test as appropriate, and presented as absolute frequencies with percentages. All ‘p’ values were two-tailed with significance defined as p < 0.05 at the level of 95% confidence interval (CI). The results were presented in the form of tables and graphs.

### Table-I: Demographic profile of the study subjects (N=50)

<table>
<thead>
<tr>
<th></th>
<th>FDRs of RA patient (n=25)</th>
<th>Healthy subjects (n=25)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years) ± SD</td>
<td>31.36 ± 10.51 (20-55)</td>
<td>31.56 ± 8.89 (20-55)</td>
<td>0.942^2</td>
</tr>
<tr>
<td>Gender distribution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>16 (64.0%)</td>
<td>14 (56.0%)</td>
<td>0.773^b</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>9 (36.0%)</td>
<td>11 (44.0%)</td>
<td></td>
</tr>
</tbody>
</table>

^aUnpaired t test was done to measure the level of significance  
^bChi-Square test was done to measure the level of significance.

Figure-1: Bar diagram showing distribution of subjects according to their age group.

Maximum participants were in age group 20-29 years in both groups of subjects followed by age group 30-39 years.

Table-II showing that anti-CCP antibody level was significantly higher in FDRs of RA patient than in healthy subjects.
Table-II: Comparison of anti-CCP antibody level between FDRs of RA patients and healthy subjects (N=50).

<table>
<thead>
<tr>
<th></th>
<th>FDRs of RA patient (n=25)</th>
<th>Healthy subjects (n=25)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-CCP level (U/mL)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td></td>
</tr>
<tr>
<td>Anti-CCP positive</td>
<td>0.50 (0.10-0.65)</td>
<td>0.09 (0.08-0.09)</td>
<td>0.002&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Anti-CCP negative</td>
<td>0.33 (0.10-0.60)</td>
<td>0.50 (0.10-0.85)</td>
<td>0.598&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Mann-Whitney U test was done to measure the level of significance
<sup>2</sup>Table-III showing that there was no significant difference in anti-CCP antibody level between male and female.

Table-III: Comparison of anti-CCP antibody level in FDRs of RA patients according to gender (n=25).

<table>
<thead>
<tr>
<th></th>
<th>Male FDRs (n=16)</th>
<th>Female FDRs (n=9)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-CCP level (U/mL)</td>
<td>Median (IQR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-CCP positive</td>
<td>0.09 (0.08-0.09)</td>
<td>0.09 (0.08-0.09)</td>
<td></td>
</tr>
<tr>
<td>Anti-CCP negative</td>
<td>0.09 (0.08-0.09)</td>
<td>0.09 (0.08-0.09)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Mann-Whitney U test was done to measure the level of significance
<sup>2</sup>Table-IV showing that 4.0% of FDRs of RA patients were anti-CCPA positive while none of healthy subjects was anti-CCPA positive.

Table-IV: Frequency of anti-CCP antibodies in FDRs of RA patients and healthy subjects (N=50).

<table>
<thead>
<tr>
<th></th>
<th>FDRs of RA patients (n=25)</th>
<th>Healthy subjects (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-CCP positive</td>
<td>1 (4.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Anti-CCP negative</td>
<td>24 (96%)</td>
<td>25 (100%)</td>
</tr>
</tbody>
</table>

Table-V showing that anti-CCP antibody level is significantly higher in FDRs of RF positive patient than in FDRs of RF negative patient.

Table-V: Comparison of anti-CCP antibody level in FDRs of RA patients according to RF in related patient (n=25).

<table>
<thead>
<tr>
<th></th>
<th>FDRs of RF positive RA patient (n=22)</th>
<th>FDRs of RF negative RA patient (n=3)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-CCP level (U/mL)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td></td>
</tr>
<tr>
<td>Anti-CCP positive</td>
<td>0.07 (0.07-0.07)</td>
<td>0.07 (0.07-0.07)</td>
<td></td>
</tr>
<tr>
<td>Anti-CCP negative</td>
<td>0.07 (0.07-0.07)</td>
<td>0.07 (0.07-0.07)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Mann-Whitney U test was done to measure the level of significance
<sup>2</sup>Table-VI showing that anti-CCP antibody level is significantly higher in FDRs of anti-CCPA positive patient than in FDRs of anti-CCPA negative patient.

Table-VI: Comparison of anti-CCP antibody level in FDRs of RA patients according to anti-CCPA in related patient (n=25).

<table>
<thead>
<tr>
<th></th>
<th>FDRs of anti-CCPA positive RA patient (n=19)</th>
<th>FDRs of anti-CCPA negative RA patient (n=6)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-CCP level (U/mL)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td></td>
</tr>
<tr>
<td>Anti-CCP positive</td>
<td>0.07 (0.07-0.12)</td>
<td>0.07 (0.07-0.12)</td>
<td></td>
</tr>
<tr>
<td>Anti-CCP negative</td>
<td>0.07 (0.07-0.12)</td>
<td>0.07 (0.07-0.12)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Mann-Whitney U test was done to measure the level of significance
<sup>2</sup>Table-VII showing that anti-CCP antibody level is significantly higher in FDRs of anti-CCPA positive patient than in FDRs of anti-CCPA negative patient.

Table-VII: Comparison of anti-CCP antibody level in FDRs of RA patients according to anti-CCPA in related patient (n=25).

<sup>2</sup>Mann-Whitney U test was done to measure the level of significance

Figure-2: A positive correlation without significance between the age of the FDRs of RA patients and the anti-CCP antibody level in them.

Pearson’s correlation was done
Figure-2: showing a positive correlation without significance between the age of the FDRs of RA patients and the anti-CCP antibody level in them.

Discussion:
Several studies have revealed that clinically symptomatic rheumatoid arthritis (RA) is preceded by a long period of autoimmunity characterized by the presence of antibodies, elevated cytokine levels, and other markers of inflammation. The factors and mechanism causing initiation of disease are not well understood. Studies of the pre-RA period are useful for understanding the pathogenesis of RA. One way to study pre-RA may be to investigate unaffected first-degree relatives of RA patients, who should share some of the genetic risk and may also share environmental risk factors for RA. Studies on anti-CCP antibodies in unaffected first-degree relatives of RA patients are very few in Bangladesh. No data have been found for the Bangladeshi population regarding pre-clinical autoimmunity, including anti-CCP seropositivity in FDRs of established RA patients prior to the development of RA. So, this cross-sectional study was conducted to see the serum anti-CCP antibody level in unaffected first-degree relatives (FDRs) of RA patients to compare with that of healthy subjects. For this purpose, 25 FDRs of RA patients and another 25 healthy subjects were enrolled as per inclusion and exclusion criteria. In this study mean age of the FDRs of RA patients was 31.36 ± 10.51 years and that of the healthy subjects was 31.56 ± 8.89 years. Minimum age of the study subject was 20 years and the maximum was 55 years in both groups. These results were supported by Smolik et al. (2013) where the mean age of FDRs was 35 ± 13 years and that of healthy control was 33 ± 11 years. In current study males were predominant in both groups of subjects. In our sociocultural condition male attendants are usually predominant in outpatient department and our sampling method was purposive and convenient as well, so males were included more in the study. Taking a large sample size enrolled by a random sampling method could overcome this limitation.
This study revealed that FDRs of RA patients have a significantly higher level of anti-CCP antibodies (within normal range). This finding was in agreement with a study done by Årlestig et al. (2011)23. These findings established that, a positive family history of RA is a risk factor for high titer of anti-CCP antibody in FDRs.

4% of FDRs of RA patients were found to be positive in present study which was comparable to a study done by Kim et al., (2013)24 on 202 FDRs from Korea and found 5% seropositivity for anti-CCP antibody. Earlier and higher expression of RA-related antibodies, especially anti-CCP antibody, may be related to increased risk for RA in the unaffected FDRs of RA patients.

Barra et al. (2013)25 worked on a Caucasian population from Southwestern Ontario, Canada and found very high prevalence of anti-CCP antibody in FDRs of RA patient (48%). Another study done by Årlestig et al. (2011)23 on a Caucasian population from Sweden revealed 21.7% anti-CCP positivity in FDRs. Kolfenbach et al., (2009)23 reported anti-CCP positivity in FDRs at a very low rate of 1.7% among the Caucasian population from the US. Another study was done by El-Gabalawy et al. (2009)26 over a North American native population from central Canada which showed 17% of FDRs to be anti-CCP positive. The rate of positive anti-CCP in this study (4%) was comparable to that in a US study (1.7%)25, but much lower than that in other studies.

The differences in the prevalence of ACP antibody in FDRs from the various studies may be due to the characteristics of the study subjects, the assays used, or the study design. It has been evident that RA is much more prevalent in some groups (eg, in Native American groups) and much less prevalent in others (eg, in southern Europe)27. For some unknown reason, in certain parts of the African continent RA has been never found28. This ethnic differences clearly had an effect on different results in different studies.

**Conclusion:**

In conclusion, this study describes unaffected first-degree relatives (FDRs) of rheumatoid arthritis (RA) patients. It had been found that anti-CCP antibody (anti-CCPA) level was significantly higher (within normal range) in FDRs of RA patient in comparison to healthy subjects, which was even more significant in FDRs of seropositive RA patient. 4% of the FDRs were anti-CCPA positive and none of the healthy subjects was positive for anti-CCPA. Anti-CCPA levels were weakly correlated with age of the FDRs. There was no significant difference in anti-CCPA level between male and female FDRs.

**Conflict of Interest:** None.

**Acknowledgement:**

We are grateful to all the teachers and staffs of the Department of Biochemistry, Dhaka Medical College and all doctors, nurses and staffs of the Department of Rheumatology, Banga bandhu Sheikh Mujib Medical University (BSMMU), Dhaka. We are also grateful to all the patients who willingly participated in our research work with their kind consent and cooperations.

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