Reliability of Anti-Cyclic Citrullinated Peptide Antibodies (Anti-CCP) for the Diagnosis of Rheumatoid Arthritis as Compared to that of Rheumatoid Factor test

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ABSTRACT

Background: Rheumatoid factor (RF) is commonly used for diagnosis of rheumatoid arthritis (RA). RF positivity is nonspecific for diagnosis of RA because it can be detected in 3-5% of normal individuals. Recent studies indicated that anti-Cyclic-Citrullinated protein (Anti-CCP) antibodies are more specific to diagnose rheumatoid arthritis and have the capacity to diagnose RA in early stages of the disease, or even before the onset of the disease when the arthritis is undifferentiated.

Objectives: This study has been conducted to assess the reliability and specificity of anti-CCP in Sudanese as an indicator of RA.

Patients and methods: In this study, patients were diagnosed as RA patients according to the American College of Rheumatology (ACR) criteria. To get further insight on the sensitivity and specificity of Anti-CCP test as compared to that of RF test in the diagnosis of RA, analysis of samples obtained was performed using enzyme linked immune sorbent assay (ELISA) and latex agglutination test. All the results were analyzed using Statistical Packages of Social Sciences (SPSS).

Results and discussion: Anti-CCP test achieved higher specificity (86.7% Vs 60%) and sensitivity (74.3% Vs 60%) compared with RF. However, despite that the accuracy of anti-CCP to diagnose RA was high (76.5%), the false negative rate reached 21.2%. Although anti-CCP test is more accurate compared with RF; it may sometimes fail to diagnose some patients with rheumatoid arthritis. However, studies on the predictive values and other conditional ratios for anti-CCP antibodies were scarce.

Keywords: Anti-CCP antibodies; Rheumatoid arthritis; Rheumatoid factor.
predictors for subsequent development of RA and joint destruction'.

Testing donors for anti-CCP antibodies is feasible nowadays in the Sudan. This study aimed to assess the reliability and specificity of anti-CCP (using ELISA kit: IgG (EUROIMMUN, Germany) in Sudanese as an indicator of RA. Blood samples were obtained from patients diagnosed by a rheumatologist as RA patients according to revised American College of Rheumatology (ACR) classification criteria for RA.

**Patients and Methods:**

**Ethical consideration:** The study had received an ethical approval from the ethical review committee at the Institute of Tropical Medicine, University of Sudan Academy. The samples were collected by well trained medical laboratory technicians.

**Study population:** Serum samples were collected from 70 patients (12 male and 58 female) who were suspected to have RA according to The American College of Rheumatology criteria. Fifteen ages matched healthy volunteers with no previous history of any type of arthritis were tested as controls.

**Anti CCP ELISA test:** Determination of the concentration of anti-CCP antibodies was performed using anti-CCP ELISA kit: IgG (EUROIMMUN, Germany). According to the manufacturer, each serum sample was diluted 1:100 into sample buffer, and 100 µl from each pre-diluted sample sera, calibrators. Positive and negative controls were pipetted into a well of micro plate. Then, the plate was incubated for 30 minutes at room temp (20-28°C). After incubation, the content of micro wells were discarded and washed three times with 300 µl washing buffer. Second incubation was conducted by adding 100 µl enzyme conjugate to each well and the plate incubated for 30 minutes at room temp. After washing (300 µl, 3x), 100 µl of substrate were added into each well, and the plate was incubated for 15 minutes at room temp. Finally, 100 µl of stop solution were added into each well and the optical density (OD) was checked by ELISA reader at 450 nm wave length. The results were expressed as ratio and the cut-off ratio was one (only Ratio >1 was considered positive).

**RF test:** RF was determined qualitatively in all samples using latex kits (Gesan, Italy). The test was carried out by adding 50 µl of the sample, 1 drop of each positive and negative control into separate circles on the black slide test. Then one drop (50 µl) of reagent was added to each circle and mixed with a stirrer. Then, slide was rotated manually for two minutes and the result was obtained by looking for agglutination. Presence of agglutination indicates positive result which indicates a RF concentration equal or greater than 8 IU/ml.

**Data Analysis:** All the results were analyzed by statistical analysis, using Statistical Packages of Social Sciences version17 (SPSS). In all of statistical tests, only P < 0.05 was considered significant.

**Results:** RF was positive in 40 % of the control group, 83.3% of which was anti-CCP negative. In comparison, only 13.3% of the control group was ACCP positive, half of them were also RF positive (table 1).

| Table-1: Detection rate of RF and ACCP in control group |
|----------------|----------------|----------------|
| Subject | ACCP Negative (%) | ACCP Positive (%) | Total (%) |
| RF-ve | 8 (53.3) | 1 (6.7) | 9 (60) |
| RF+ve | 5 (33.3) | 1 (6.7) | 6 (40) |
| Total | 13 (86.7) | 2 (13.3) | 15 (100) |

Regarding test group, 60% of the patients were RF positive; only 11.9% of them were anti-CCP negative. In comparison, 74.3% of the patients were anti-CCP positive, 28.8% of them were RF negative (table 2).

| Table-2:Detection rate of RF and ACCP in patients with rheumatoid arthritis |
|----------------|----------------|----------------|
| Subject | ACCP Negative (%) | ACCP Positive (%) | Total (%) |
| RF-ve | 13 (18.6) | 15 (21.4) | 28 (40) |
| RF+ve | 5 (7.1) | 37 (52.9) | 42 (60) |
| Total | 18 (25.7) | 52 (74.3) | 70 (100) |
Testing for conditional ratios, assessment of rheumatoid arthritis activity using anti-CCP as a disease marker is proved to be more sensitive (74.3% Vs 60%) and specific (86.7% Vs 60%) when compared with RF. Positive predictive value (PPV) (96.3% Vs 87.5%) and negative predictive value (NPV) (41.9% Vs 24.3%) were superior in case of ACCP. These findings make anti-CCP more accurate determinant of rheumatoid arthritis (76.5% Vs 60%) when compared with RF (table 3 and 4).

Table-3: Detection rate of rheumatoid factor in patients with rheumatoid arthritis

<table>
<thead>
<tr>
<th></th>
<th>Rheumatoid Arthritis</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF</td>
<td>+ve</td>
<td>42</td>
<td>6</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>-ve</td>
<td>28</td>
<td>9</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>70</td>
<td>15</td>
<td>85</td>
</tr>
</tbody>
</table>

Pearson Chi-Square value = 2.010
P = 0.156
Sensitivity = 60%
Specificity = 60%
Positive predictive value = 87.5%
Negative predictive value = 24.3%
Accuracy = 60%

Table-4: Detection rate of rheumatoid factor in patients with Rheumatoid Arthritis

<table>
<thead>
<tr>
<th></th>
<th>Rheumatoid Arthritis</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACCP</td>
<td>+ve</td>
<td>52</td>
<td>2</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>-ve</td>
<td>18</td>
<td>13</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>70</td>
<td>15</td>
<td>85</td>
</tr>
</tbody>
</table>

Pearson Chi-Square value = 19.808
P = 0.000
Sensitivity = 74.3%
Specificity = 86.7%
Positive predictive value = 96.3%
Negative predictive value = 41.9%
Accuracy = 76.5%

Discussion:
In this study, anti-CCP test achieved higher specificity (86.7% Vs 60%) and sensitivity (74.3% Vs 60%) compared with RF.

The specificity of a test is the probability that a test will produce a true negative result when used in control group while sensitivity is the probability that the test will produce a true positive result when used in patients (test group)\(^8,9\). Accordingly, it seems that ACCP is better, when compared with RF, to rule out rheumatoid arthritis in healthy people and to confirm the disease in patients with the disease.

Regarding predictive values, anti-CCP test achieved 96.3% PPV compared with 87.5% in case of RF. Regarding NPV, Both anti-CCP and RF tests attained poor performance (41.9% and 24.3% respectively). PPV refer to the probability that a person is diseased when the test is positive while NPV is the probability that a person is not diseased when the test result is negative\(^10\). As shown in table 2, anti-CCP test failed to diagnose 25.7% of patients. This explains the relatively lower scores of NPV. However, it is worth mentioning that in practice, predictive values should only be calculated from cohort studies or studies that legitimately reflect the number of people in that population who have the disease of interest at that time. This is because predictive values are inherently dependent upon the prevalence of the disease.

In concordance with the results of previous studies, our findings of sensitivity and specificity for anti-CCP antibodies in RA are approximately in agreement with the studies that reported a sensitivity and
specificity for RA\textsuperscript{11}. However some other studies utilized CCP2 kit confirmed the higher specificity of the test \textsuperscript{7, 12}.

**Conclusion**
The high specificity of anti-CCP antibodies is particularly useful in RF-negative RA patients. Anti-CCP antibodies are more specific than RF for diagnosing rheumatoid arthritis. In Sudanese patients with RA, anti-CCP antibodies exhibit a better diagnostic value than RF.

**References:**