Comparison of Rheumatoid Factor and anti-Cyclic-Citrullinated protein antibodies for the Diagnosis of rheumatic arthritis in

Khartoum, Sudan

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Abstract

Background: The objective of the current study was to determine the sensitivity and specificity of anti-Cyclic-Citrullinated protein antibodies (anti-CCP antibodies) as compared to that of Rheumatoid Factor (RF) in diagnosing patients with rheumatoid arthritis (RA). Methodology Fifty six samples also were collected from Sudanese patients (46 females, 9 males) with rheumatic diseases who visited the rheumatology clinic ElRibat Hospital, Khartoum, Sudan. Titers of RF and anti-CCP antibodies of each patient were recorded. Sensitivity and specificity of the test were evaluated using ELISA as the gold standard method. Results The sensitivity of (RF) test (41/56) was 73.2% whereas the sensitivity of Anti CCP test (34/56) was 60.7%. The specificity of RF test (44/56) was 78.6%, whereas the specificity of Anti CCP test (54/56) was 96.4%. Conclusions The combination of anti-CCP and RF tests provides nearly 100% and thus could be helpful in the differential diagnosis of RA and other rheumatic diseases.

Keywords: Anti-CCP antibodies, Rheumatoid Factor, Rheumatoid arthritis.

 dumpstersiddle:الخلفية:  يهدف من الدراسة الحالية هو تحديد حساسية ونوعية ( الأجسام المضادة لل CCP) في تشخيص المرضى الذين يعانون من التهاب المفاصل الروماتويدي (RA). طرق البحث تم جمع ست وخمسون عينة أيضاً من المرضى السودانيين ( 46 إناث و 9 ذكور) يعانون من أمراض روماتيزمية والذين زاروا عيادة الروماتيزم مستشفى الرباط ، الخرطوم، السودان. تم تسجيل معايرة الأجسام المضادة لأختبار العامل الروماتويدي والأجسام المضادة ل كل CCP. تم تدقيق الحساسية والنوعية باستخدام الأنتيزم المناعي الملتصق كمعيار قياسي. النتائج كانت حساسية اختبار 73.2% (RF) في حين بلغت حساسية مكافحة CCP 70.7% . كانت خصوصية اختبار 6% (RF) في حين كانت خصوصية مكافحة CCP 6% . وكانت خصوصية اختبار CCP 96.4% . الاستنتاجات مزيج من مكافحة RF بالاختبارات يوفر ما يقرب من 100% . وبالتالي قد تكون مفيدة في التشخيص الفرعي ل التهاب المفاصل الروماتويدي و غيرها من الأمراض الروماتيزمية.
Introduction:

Rheumatoid arthritis (RA) is an inflammatory disease which, though systemic, typically involves the small joints of the hands and feet, often symmetrically. It affects approximately 1% of the population and is more common in women [1]. The etiology of RA is not fully understood [2]. It is clear that both genetic and environmental factors play important roles [3]. RA is the most commonly diagnosed systemic inflammatory arthritis. Women and those with a family history of the disease are most often affected. Criteria for diagnosis include having at least one joint with definite swelling that is not explained by another disease [4]. The prognosis for patients with rheumatoid arthritis has improved dramatically over the past two decades. The reasons for the improved prognosis include earlier diagnosis, treatment targeted to low disease activity or remission [5]. Laboratory investigation can improve diagnostic sensitivity and specificity in relatively early rheumatoid arthritis [6].

The diagnosis of RA is primarily based on clinical symptoms, so it is often difficult to diagnose RA in very early stages of the disease [7]. For many years rheumatoid factor has been widely used in the diagnosis of RA apart from clinical manifestations. However RF is also present in other rheumatic diseases and in a proportion of healthy individual, makes it is not very specific for RA. Laboratory diagnosis of RA in Sudan was dependent on RF. Recently; anti-CCP took place in the diagnosis of RA together with RF. In addition to its high specificity, it can enable clinicians to effectively distinguish RA patient from other RA resembling diseases. It can predict the outcome of early RA as it has prognostic ability for the future development of RA and assist in planning therapeutic strategy.

The objective of the current study was to determine the sensitivity and specificity of anti-cyclic citrullinated peptide (anti-CCP) antibodies as compared to that of RF in diagnosing patients with Rheumatoid Arthritis.

Materials and methods:

Fifty six Sudanese patients (46 females, 9 males; mean age ± SD, 46.0 ± 11.7 years), with rheumatic diseases who visited the rheumatology clinic Al Ribat Hospital, Khartoum – Sudan. From 2002 to 2004 were enrolled in this study. All of the selected patients fulfilled 1987 American College of Rheumatology (ACR) Criteria for RA were included in the RA group. The other fifty six patients were
classified as a control group (27 females, 28 males; mean age ± SD, 37.4 ± 17.2 years). 60 Samples also were collected from apparently healthy of first degree relatives of patients with rheumatoid arthritis. The demographic data, titers of RF and anti-CCP antibodies of each patient were recorded. All serum samples were obtained and stored at –80°C until assayed. The study was approved by the Ethics committee of El Neelain University. Serum from each subject was tested for anti-CCP antibodies and by enzyme linked immunosorbent assay (ELISA) and RF (IgM) latex test. Sensitivity and specificity of the test were evaluated using the clinical diagnosis as the gold standard.

Statistical analyses were performed using the biomedical Stats Direct Statistical Software v2.7.9 (7/9/2012). The conventional 5% level of significance was used for all statistical tests. The confidence interval (CI) shown for any estimate is the 95% CI.

The study was approved by the Ethical committee Board Al Neelain University, Khartoum, Sudan.

Results:
A total of fifty six cases, fifty six controls and sixty healthy apparently relatives were enrolled. The distribution of Age and sex among the three different groups was shown in (Table.1) and (Figure.1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Age in years</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>37 ±17.2</td>
<td>46 ±13.7</td>
<td></td>
</tr>
<tr>
<td>RA Cases</td>
<td>46 ±11.7</td>
<td>52 ±14.7</td>
<td></td>
</tr>
<tr>
<td>Relatives</td>
<td>31 ±14.3</td>
<td>33 ±16.1</td>
<td></td>
</tr>
</tbody>
</table>
In a paired test design, each individual in the three groups was subjected to two tests; anti-CPP and RF. The results were dichotomized (positive/negative). Anti-CCP results were also available in continuous scale. The dichotomized results of the two tests are shown in (Table.2).

Table2. Anti-CCP and RF test results in controls, cases and relatives.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of subjects</th>
<th>Anti CCP Test</th>
<th>RF Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Controls</td>
<td>56</td>
<td>2</td>
<td>54</td>
</tr>
<tr>
<td>RA Cases</td>
<td>56</td>
<td>34</td>
<td>22</td>
</tr>
<tr>
<td>Relatives</td>
<td>60</td>
<td>9</td>
<td>51</td>
</tr>
<tr>
<td>Total</td>
<td>172</td>
<td>45</td>
<td>127</td>
</tr>
</tbody>
</table>

Figure 1. Distribution of study subject according to their gender.

In a paired test design, each individual in the three groups was subjected to two tests; anti-CPP and RF. The results were dichotomized (positive/negative). Anti-CCP results were also available in continuous scale. The dichotomized results of the two tests are shown in (Table.2).
Table 3 Comparison of anti-CCP and RF test results in the three groups

<table>
<thead>
<tr>
<th>Group</th>
<th>RA cases</th>
<th></th>
<th>Controls</th>
<th></th>
<th>Relatives</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACCP test</td>
<td></td>
<td>ACCP test</td>
<td></td>
<td>ACCP test</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ve -ve</td>
<td>Total</td>
<td>+ve -ve</td>
<td>Total</td>
<td>+ve -ve</td>
<td>Total</td>
</tr>
<tr>
<td>RF test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ve</td>
<td>29 12</td>
<td>41</td>
<td>0 12</td>
<td>12</td>
<td>3 7</td>
<td>10</td>
</tr>
<tr>
<td>-ve</td>
<td>5 10</td>
<td>15</td>
<td>2 42</td>
<td>44</td>
<td>6 44</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td>34 22</td>
<td>56</td>
<td>2 54</td>
<td>56</td>
<td>9 51</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 3 shows the results of anti-CCP and RF tests were both correctly positive on only 29/56 (52%) of RA cases, and were both correctly negative on 42/56 (75%) of controls. The table also shows that while the two tests were both falsely positive on 10/56 (18%) of RA cases, they did not falsely pick out any of the controls as positive. Anti-CCP correctly identified only 34/56 (61%) compared with 41/56 (73%) correctly identified by the RF test. Anti-CCP however, correctly identified 54/56 (96%) of the controls as RA negatives compared to only 44/56 (79%) correctly identified by RF as RA negatives.

In relatives of RA cases, anti-CCP an RF tests agreed upon 3/60 (5%) as RA positives and 44/60 (73%) as RA negatives. The two tests disagreed on 13/60 (22%) of relatives of the RA cases; 6 were deemed positive by the anti-CCP test and negative by the RF test; and 7 were deemed positive by the RF test and negative by anti-CCP test. The anti-CCP2 antibodies were positive in two relatives of RA patients.

The sensitivity of RF test (41/56) was 73.2% (CI: 59.7% to 84.2%) whereas the sensitivity of Anti CCP test (34/56) was 60.7% (CI: 46.8% to 73.5%). The specificity of RF test (44/56) was 78.6% (CI: 65.6% to 88.4%), whereas the specificity of Anti CCP test (54/56) was 96.4% (CI: 87.7% to 99.6%) as shown in (Table.3).

The parallel interpretation scheme has a sensitivity of 82.1%, and specificity of 75%, while the serial interpretation scheme has a sensitivity of 51.8% and a specificity of 100%. The sensitivity of the combined test (82.1%) is higher than that of either of the two single tests (60.7% for ACCP and 73.2% for RF).
specificity of the combined test (100%) is also higher compared with that of either anti-CCP test (96.4%) or RF test (78.6%) (Figure 2).

Figure 2. Proportionate reduction in uncertainty (PRU) for combined test (parallel interpretation scheme)
Given a prevalence of 50%, the post-test likelihood of RA for a positive anti-CCP test result is 94.4% (CI: 81.3% to 99.3%) compared with 77.4% (CI: 63.8% to 87.7%) for RF. The change over pre-test likelihood for a positive anti-CCP test is equal to 44.4% compared with 27.4% for RF.
Table 4. Comparison of performance measures of RF, anti-CCP and the combined test

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>LR+</th>
<th>LR-</th>
<th>DOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF</td>
<td>73.2%</td>
<td>78.6%</td>
<td>77.4%</td>
<td>74.6%</td>
<td>0.42</td>
<td>-</td>
<td>10.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(2.09, 5.88)</td>
<td>-</td>
<td>(3.9 - 26.4)</td>
</tr>
<tr>
<td>Anti-CCP</td>
<td>60.7%</td>
<td>96.4%</td>
<td>94.4%</td>
<td>71.1%</td>
<td>17</td>
<td>-</td>
<td>41.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(4.92, 62.44)</td>
<td>-</td>
<td>(9 - 374.3)</td>
</tr>
<tr>
<td>Combined (Parallel)</td>
<td>82.1%</td>
<td>75%</td>
<td>76.7%</td>
<td>80.8%</td>
<td>3.286</td>
<td>-</td>
<td>13.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(2.13–5.363)</td>
<td>-</td>
<td>(5.1 - 38.4)</td>
</tr>
<tr>
<td>Combined (Serial)</td>
<td>51.8%</td>
<td>100%</td>
<td>100%</td>
<td>67.5%</td>
<td>∞</td>
<td>-</td>
<td>∞</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(8.034, ∞)</td>
<td>-</td>
<td>(13.6 - ∞)</td>
</tr>
</tbody>
</table>

PPV: Positive Predictive Value, NPV: Negative Predictive Value, LR: Likelihood Ratio, DOR: Diagnostic Odd Ratio

Discussion:

Our findings have indicated that, the sensitivity of RF was moderately high (73.2%), this finding is in agreement with most of studies which resulted in sensitivity about 80% (11, 12). Anti-CCP showed moderate sensitivity (60.7%), however there is considerable variation in diagnostic sensitivity of Anti-CCP among studies, ranging from 41%–80% (13, 14). The wide range of anti-CCP sensitivity may contributed to the variation of the cut-off value used to define a positive result (15), anti-CCP antibodies measurements weren't followed-up(16), the ethnicity of patients(17) and the complexity of RA disease (18).
The anti-CCP test achieved higher specificity (96.4% Vs 78.6%) and low sensitivity (%60.7% Vs 73.2%) compared to RF. The high specificity obtained for anti-CCP in the current study is comparable to those obtained by other studies (Serdaroğlu et al, Agyei- Frempong et al and Zeng et al) which reported a specificity of 97% , 96.69% and 100% respectively (17,19,20). Our results also in accordance with another study conducted in Singapore showed that the specificity and sensitivity of anti-CCP antibodies were 92.1% and 62.3% respectively (21). However, few studies showed similar in specificity but different in the sensitivity of anti-CCP antibodies to this study. A cohort study in Germany by Sauerland et al demonstrated specificity of 94.5% and slightly better sensitivity (74.0%) compared to this study (22). The difference in the sensitivity may attribute to the difference in the assay used and patient’s selection in the study.

Our study revealed that the combined measurement of both anti-CCP and RF antibodies resulted in improved specificity (100%) and sensitivity (51.79%), also PPV were higher (100%) than for testing of either auto-antibody alone. Our results are in accordance with results obtained by several studies (19,23,24). The low sensitivity of anti-CCP test has indicated that a negative anti-CCP antibodies does not exclude the disease, but its high specificity means that a positive result may increase the probability that the patient will have RA.

Our study revealed that 17.2% of RF negative tested patients were positive for anti-CCP antibodies. The positive anti-CCP results especially in sero-negative RA patients strongly supported the diagnosis of RA serologically. This result was similar to the previous studies (24-26). It was also noted that both anti-CCP and RF were negative in 17.8 % of the patients involved in this study, comparably; a previous study by Inanc et al (27) demonstrated that 30% of RA patients were negative for the two tests. This suggests that a still unknown etiopathological event associated with the development of RA.

The positive predictive value in this study was good, 94.4% and negative predictive value was 71.0%. Also 94.4% would be diagnosed to have RA based on ACR criteria among those individual with a positive anti-CCP antibodies. This finding was slightly higher than a study by Lee and Schur that gave a positive predictive value of 82.9% (28).

In a study conducted in Sudan, Bolad et al found that the 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) (29). Our results were agree with the PPV but we observed good performance of NPV (76.4% and 71.1% respectively).

Our study demonstrated that autoantibody testing may be useful for predicting RA development in individuals in high-risk populations. RantapD-Dahlgvist et al also found the prevalence of antibodies more than 1.5 years prior to disease onset was 33.7% with anti-CCP, 19.3% with IgM-RF, 33.7% with IgA-RF, and 16.9% with IgG-RF these results were all highly significant compared to matched controls (30). We also demonstrated a significant positivity of anti-CCP2 in relatives of RA patients and diagnosed RA in two of them. The dominance of females was noticed in the current study, the result is in accordance with the result obtained by Teh and Wong, 84.4% of the RA patients were female (31). The use of anti-CCP and RF tests combined is considered to be the ‘gold standard’ in the detection of RA, because the combination of them provides nearly 100% and thus could be helpful in the differential diagnosis of RA and other rheumatic diseases. In addition, this test may be very influential for the rheumatologists to choose the best therapeutic strategy in patients with recent-onset arthritis.

As early treatment of RA is important for providing the patient with the best outcome and quality of life; our study was pioneering in the clinical assessment of the association of both anti-CCP antibodies and RF in RA patients and their relatives in Sudan.

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References:


