The Diagnostic Value of Anti-Cyclic Citrullinated Peptide Antibodies in Patients with Rheumatoid Arthritis

Shabaan Hashem, Adel Mahmoud, Hossam Fahmy, Reem Habeib, Sara Eltigani

Department of Internal Medicine & Rheumatology, Faculty of Medicine, Assuit University, Faculty of Medicine, Ain shams university and Faculty of Pharmacy, Bany Sweif University, Egypt.

Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic inflammation of the joints. The aim of this study was to evaluate the diagnostic value of anti CCP antibodies in patients with RA. This study included three groups; group I: 60 early diagnosed RA patients selected according to the ACR, group II: 30 patients of arthritis other than RA, group III: 10 healthy age and sex matched volunteer. Sera from all participants were tested for rheumatoid factor (IgM RF) and anti-CCP IgG antibodies using ELISA. Of 60 patients in group I, 52 subjects (86.7%) were RF positive, whereas all participants in groups II and III were negative. Similarly 56 patients (93.3%) of group I were anti-CCP antibody positive, but all subjects in groups II and III were negative. Thus detection of anti-CCP antibodies can be used as a diagnostic indicator for patients with early RA (P<0.0001).

Rheumatoid arthritis is a common rheumatic disease. About 1% of the world's population is affected by RA (O'Dell, 2005). The misdirected immune system activation causes the body to attack its own tissues. This leads to the inflammation of the tissues lining of the joints and also the inflammation of the synovium, which grows into a thick abnormal tissue leading to the destruction of the cartilage and the underlying bone surrounding the joint, ligaments and tendons and can eventually lead to deformed joints (William et al., 2003). Researchers suggest that in the joints of RA patients proteins may be changed into citrulline as part of the process that leads to inflammation of the rheumatoid joint. This is present in the blood of most patients with RA and is used in the diagnosis of RA when rheumatoid factor is not present (Gerard et al., 2000). The early diagnosis of RA has become a priority owing to the availability of effective disease-modifying agents that can improve patient wellbeing and influence the clinical outcome (Bizzaro, 2007). Moreover, there is increasing evidence that the first few months of disease represent a unique therapeutic opportunity and that such early therapeutic intervention is crucial in preventing irreversible joint damage. So, it is widely accepted that early and accurate diagnosis of RA is critical in disease management (van venrooij et al., 2006).

Nikolaisen et al., (2007) stated that we require a more reliable disease marker than the non-specific IgM rheumatoid factor (RF) test. However this represents a real challenge, as there was no available pathognomonic clinical, radiological, or immunological features (Bizzaro; 2007).

Within the last few years a growing number of publications (Bizzaro; 2007) have reported that the second generation anti-cyclic citrullinated peptide (anti-CCP2) test may become the marker of choice for diagnosing early RA. Detection of anti-CCP2 appears to be highly specific for the disease with sensitivity comparable to the widely used but
The diagnostic value of anti-ccp antibodies in patients with RA

less specific RF test. Additionally, positive anti-CCP2 can predict future development of RA in both symptomatic individuals and in patients with undifferentiated arthritis. Furthermore, antibody levels may correlate with progression to erosive disease (van venrooij et al., 2006).

Consequently the present study was designed to evaluate the diagnostic value of anti-CCP2 antibody assay.

Subjects and Methods

Subjects

This study included 90 individuals, divided into 3 groups:

Group I

Included 60 patients with Rheumatoid Arthritis, selected according to the American College of Rheumatology (ACR) criteria for diagnosis of Rheumatoid Arthritis (Arnett et al., 1998).

Group II

Included 30 patients with arthritis other than Rheumatoid Arthritis. Ten patients with Systemic Lupus Erythematosus [Group II S], selected according to the ACR criteria for diagnosis of SLE (Arnett et al., 1998); 10 patients with Osteoarthritis [Group II O], selected according to the ACR criteria for diagnosis of OA (Altman et al., 1986); and 10 patients with Gout [Group II G]

Group III

Included 10 healthy volunteers matched for age and sex.

All patients and controls were selected from the out-patient clinic and in-patient ward of the Rheumatology Department in Ain Shams University hospital. A verbal informed consent was obtained from all subjects. An aliquot of 5ml venous blood were collected from each subject, centrifuged and the serum was stored at -5 °C until used.

Methods

- Full medical history
A detailed history was obtained from all subjects with special emphasis on symptomatology of RA, SLE, Osteoarthritis and Gout.

- Thorough clinical examination
All patients were subjected to full general examination and local musculoskeletal examination including signs of arthritis, swelling, deformity, power and range of movement of joints.

- Laboratory assessment
These included immunological testing of:

  - Rheumatoid Factor (IgM) was detected using an ELISA kit (QUANTA Lite™ IgM RF ELISA kit, INOVA Diagnostics, Inc.) according to the manufacturer instructions.

  - Anti-CCP antibodies were detected using an ELISA kit (QUANTA Lite™ CCP2 ELISA kit, INOVA Diagnostics, Inc.) according to the manufacturer instructions.

Interpretation of the results

The samples were classified as negative, weak positive, moderate positive or strong positive according to the following ranges:

- Positive: A positive result indicated the presence of IgG anti-CCP antibodies and suggested the possibility of RA.

- Negative: A negative result indicated absence of IgG anti-CCP antibodies or levels below the assay cut-off.

Statistical Analysis

Analysis of data was done using SPSS (statistical program for social science). Description data included quantitative variables as mean, Standard Deviation (SD) and range; and qualitative variables, numbers and percentages. Chi- square test was used to compare qualitative variables while unpaired t-test was used to compare two independent groups. Mann Whitney test was used instead of unpaired t-test in non parametric data. In all tests considered significant at P <0.05.

Results

Group I (RA) showed RF positivity of (86.7%) (P=0.000) when compared to the two other groups which showed RF negative (Table 1).
Group I (RA) showed anti-ccp positivity of (93.3%) \( (P=0.000) \) when compared to the two other groups which showed anti-ccp negative (Table 2).

CCP is an indicator for patients of group I (RA patients) since it is positive for group I, negative for groups II and III (Table 3).

A positive result indicates the presence of anti-CCP antibodies and suggests the possibility of Rheumatoid arthritis. A negative result indicates no CCP antibodies.

In group I when comparing RF with CCP it showed significant difference \( (P=0.000) \) which indicated that CCP is a strong indicator for RA (Table 4).

<table>
<thead>
<tr>
<th>RF (IgM RF)</th>
<th>positive</th>
<th>negative</th>
<th>total</th>
<th>*P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (RA)</td>
<td>52</td>
<td>8</td>
<td>60</td>
<td>0.000</td>
</tr>
<tr>
<td>Group II (SLE, OA, GA)</td>
<td>0</td>
<td>30</td>
<td>30</td>
<td>0.000</td>
</tr>
<tr>
<td>Group III (CONTROL)</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>0.000</td>
</tr>
</tbody>
</table>

\*P<0.05 is significant.

Table 2. IgG anti-CCP antibody rates among the different study groups

<table>
<thead>
<tr>
<th>CCP</th>
<th>positive</th>
<th>negative</th>
<th>total</th>
<th>*P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (RA)</td>
<td>56</td>
<td>4</td>
<td>60</td>
<td>0.000</td>
</tr>
<tr>
<td>Group II (SLE, OA, GA)</td>
<td>0</td>
<td>30</td>
<td>30</td>
<td>0.000</td>
</tr>
<tr>
<td>Group III (CONTROL)</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>0.000</td>
</tr>
</tbody>
</table>

\*P<0.05 is significant.

Table 3. Range of anti-CCP IgG antibody units in the study groups

<table>
<thead>
<tr>
<th>CCP</th>
<th>Range (in units)</th>
<th>Mean ± SD</th>
<th>*P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (RA)</td>
<td>12.0 – 380.0</td>
<td>118.918 ± 99.352</td>
<td>0.000</td>
</tr>
<tr>
<td>Group II (SLE, OA, GA)</td>
<td>9.0 – 32.0</td>
<td>16.233 ± 3.910</td>
<td>0.000</td>
</tr>
<tr>
<td>Group III (CONTROL)</td>
<td>4.0 – 14.0</td>
<td>9.111 ± 3.516</td>
<td>0.000</td>
</tr>
</tbody>
</table>

\*P<0.05 is significant.

Table 4: Cross tabulation of RF (IgM) and anti-CCP IgG antibodies among Group I (RA)

<table>
<thead>
<tr>
<th>IgM RF</th>
<th>CCP</th>
<th>positive</th>
<th>negative</th>
<th>*P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>52</td>
<td>0</td>
<td>52</td>
<td>0.000</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>0.000</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>4</td>
<td>56</td>
<td>0.000</td>
</tr>
</tbody>
</table>

\*P<0.05 is significant.

**Discussion**

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. A disease specific autoantibody that could be used as a serological marker would therefore be useful (Martinus et al., 2002).

Our study showed that detection of anti-CCP antibodies represents a strong indicator for patients with early rheumatoid arthritis. This finding is compatible to these of (Martinus et al., 2002) who concluded that the antibody response directed to citrullinated antigens has the most valuable diagnostic and prognostic potential to rheumatoid arthritis.

Our study showed that CCP is a better indicator for rheumatoid arthritis when compared to IgM RF which was compatible
with the study of (Gerard et al., 2000) who reported that the detection of anti-CCP by ELISA might be very useful for diagnosis and therapeutic strategies in rheumatoid arthritis of recent onset. Their study reported sensitivity of 96% for CCP versus 91% for IgM RF (P= 0.016).

However, our data were different from these reported by Bizzaro et al., 2001 and Alf et al., 2004. The study of (Bizzaro et al., 2001) stated that rheumatoid factor had a higher sensitivity 62% than anti-CCP which had a sensitivity of 41%. Their data are different than our findings.

The Alf et al., 2004 study claimed that the anti-CCP antibody assay has a similar diagnostic sensitivity to that of rheumatoid factor in early rheumatoid arthritis, but is better as a predictor of the disease course over 3 years, while our research showed that anti-CCP is better than rheumatoid factor in early diagnosis of rheumatoid arthritis.

Our research concluded that anti-CCP is a good predictor for the disease since 93.3% of the patients with rheumatoid arthritis had positive anti-CCP. Another study (Bas et al., 2002) used ELISA kits purchased from eurodiagnostica reported that anti-CCP antibodies can be useful in establishing the diagnosis of rheumatoid arthritis with similar high sensitivity of 96%.

Based on our results, we concluded that the presence of anti-CCP IgG antibodies is a useful diagnostic marker for RA.

References


