Incidence of psoriatic arthritis in Iraqi psoriatic patients according to serological tests

Wisal Salman Abd
Department of Biotechnology, College of Science, University of Baghdad, Iraq.

Abstract
The present study was accomplished on 82 patients 52 with psoriasis and 30 with psoriatic arthritis (PsA). Age of incidence was 48 years, 42 were female with mean age 39 years and 40 were male with mean age 42 years. Anti-CCP antibodies were estimated using ELISA technique while rheumatoid factor (RF) and C-reactive protein (CRP) were tested using latex serological methods. Ten (50%) PsA patients was positive with Anti-CCP while 5 (16.7%) patients were positive with RF. 1 cutaneous psoriatic patient (1.9%) had positive Anti-CCP and no patients with cutaneous psoriasis had positive RF. RF can help in distinguishing between RA and psoriasis. Seven (23.3%) PsA patients were followed-up for Anti-CCP antibodies for 1 year every 3 months. There was an observed increased in levels of Anti-CCP antibodies. It could be concluded that Anti-CCP antibody is a good indicator for the follow up study and progression of PsA.

Keyword
Psoriatic arthritis, psoriasis, anticcp.

Introduction
Psoriatic arthritis is complex disease; its complexity comes from the pathogenesis and physiology of the disease. It is an incapacitating illness that causes inflammation in joints in 25% of patients with psoriasis. Psoriasis is a rather common papulosquamous disorder.1-3 Advanced research in immunology open new prospect in therapies. There is no good test to diagnose PsA in earliest stages.4 The peak age incidence is 30-50 in both male and female.2 After ten years of skin disease, patients develop joints manifestations, less than 20% have joint disease before skin manifestations. Symptoms vary in severity. There are different clinical types "monoarthritis of the large joints, distal interphalangeal arthritis, spondylarthritides, or a symmetrical deforming polyarthropathy more akin to that of rheumatoid arthritis". If patients do not take treatment the inflammation could be persist and leads to complete inabilities.5 The most important clinical picture is the "dactylitis" (which is inflammation of the tendon combined with inflammation of joints and all digits). At the inclusion loci inflammation could be appear in "plantar fascia, rib, spine and pelvis". The severity of cutaneous disease does not correlate with that of the joints. Previous studies proved that "nail psoriasis" is associated with PsA although recent studies changed these thoughts.7 Other symptoms are; "conjunctivitis, iritis and urethritis". Great advances occur in the last ten years in immunology, genetics and epidemiology of PsA and new aspects in development of drugs. To avoid irreversible joints ruining, early diagnosis by advanced techniques is very necessary.8 A study showed that the most important test for early diagnosis is MRI in addition to clinical criteria.8 Other supporting tests include, total immunoglobulin G increased interleukin 23.9 Others showed that clinical signs of psoriasis and PsA have no
association with the above laboratory tests.\textsuperscript{10} The same authors suggested another serological test that show an association between PsA and cutaneous psoriasis.\textsuperscript{11} A good prognosis for PsA is early diagnosis in patients.\textsuperscript{12} There are two indices for diagnosis: One is mCDPAI; this index depends on four items "joints, skin, dactylitis and enthesitis", the second index is AMDF which is "skin and joints" and VAS assessment.\textsuperscript{13} The reason for the present study is to study the relationship between immune diseases and the resulting problems in the joints and for follow-up.

**Comorbidities** Sommer et al. in 2006 and Kogan et al. in 2012 found that patients with higher weight and larger waist line had a bad prognosis and more likely recur after two years.\textsuperscript{14,15} Others believed that patients with PsA on low caloric diet and fibers showed good prognosis MDA.\textsuperscript{17} Severe heart failure occurs to PsA patients with imbalance in thyroid function,\textsuperscript{18} while other studies stated that there was a decrease in the percent of heart failure in the same patients when they used TNF blockers. In other paper increasing the incidence of heart failure in psoriatic patients with arthritis.\textsuperscript{19} Lloyd and his co workers stated that Psoriatic arthritis (PsA) is a chronic inflammatory disease in which arthritis is associated in most cases with psoriasis. The biological and clinical spectrum of PsA may present common elements with rheumatoid arthritis (RA; e.g. symmetrical arthritis of the hands, elevated acute phase proteins) or with the general class of spondylarthropathies (e.g. dactylitis, enthesitis, sacroiliitis).\textsuperscript{3}

**Immunopathology** Innate and acquired immune response have a role in PsA.\textsuperscript{20,21} Predomination of T-helper 1 cells produce gamma interferon at the site of skin lesions. Interferon alpha, T-lymphocyte and dendritic cells play a crucial role in the pathology of psoriasis.\textsuperscript{5,22} Another helper cells called T-helper 17 have a very important role in the pathogenesis of joints and cutaneous disease.\textsuperscript{23} TNF, interferon-alpha, interferon-gamma, interleukin-6 and interleukin-1 alpha promote production of interleukin-12 that activate T helper 1 cells and interleukin -23 activate T helper - 17 cells.\textsuperscript{24} Then interleukin-17 secreted from T helper-17 cell, different cytokines are produced and after series of events "arthritis" occurs, this leads to production of many cytokines from T helper-1 like tumor necrosis factor, interleukin-1β and interleukin - 10 that are found in syanovium of patients with PsA. The presence of above cytokines in addition to interleukin-17 and interleukon-23 are indicators for "osteoelastogenesis and bone erosion".\textsuperscript{25-27} Production of those cytokines inside the cells promote production of a regulator of natural immune response which is called (nuclear factor kappa β) "NFKβ".\textsuperscript{28} This factor causes activation of genes responsible for different immune diseases like PsA.\textsuperscript{21} "RANK AND RANKL" are receptor activators for "NFKβ and NFKβ ligand" which are expressed under the effect of tumor necrosis- α. The activity of them is to stimulate formation of osteoclast building blocks in synovial tissue, the presence of T- lymphocyte infiltration as a result of inflammation caused by disease pathogenesis. Over-regulation of osteoclast leads to bone resorption.\textsuperscript{29}

Psoriasis and other Autoimmune Diseases: According to many researches there is a strong correlation of autoimmune diseases with each other.\textsuperscript{30-33} Psoriasis has an association with other autoimmune diseases.\textsuperscript{34-37}

Genetics: Recent studies prove there are important differences in genetic between PsA and psoriasis. Inheritance of PsA is three to four times more than psoriasis. Many genes have specificity for PsA but not for psoriasis like MICA *002.
HLA-C associated in expression of PsA. Different genes are linked to protein "(TrAF3)" have an important role in the pathway of T helper-17. Other genes are associated with NFkB activities which regulates transcription of different genes associated with PsA. Another gene polymorphism is VEGF C(-2578)A associated with PsA.

**Laboratory Tests** Till now researchers couldn’t find a test for diagnosis of PsA. Rheumatoid factor (RF) provide a designation between RA and PsA as two different disorders, anticitrullinated antibody have a crucial role in diagnosis of RA. Those antibodies were produced by plasma cells in the synovial fluid where it is bound to protein there exactly to the antigenic determinants that include citrulline. The criteria of RA classification include AntiCCP Abs. Estimation of anticcp Ab titer benefit in early diagnosis and evaluation of activity of RA especially in women. The objective of the present work using some serological parameters in PsA and psoriasis. The present study aimed to find the incidence of PsA in patients with Psoriasis in Iraq.

**Patients and Methods**

**Patients** A total of 96 persons were included in this study: 82 patients with psoriasis and 14 healthy individual were included in this study. All patients were admitted to Alfanar medical laboratory and some other private medical laboratories from September 2017 to September 2018 (samples were selected depending on doctor's diagnosis), age of the patients and control was range 15-73 years.

**Sample collection** Three milliliters of blood was withdrawn from patients and control by vein puncture. Sera was separated by centrifuge at 3000 rpm for 15 minutes. Samples were kept in the freezer at -4°C until ELISA assay performance.

**ESR** Accomplished by Westergren method.

**Rheumatoid Factor and C reactive protein antibodies** These tests were performed by serological method (LiNEAR) RF- LATEX. One drop of unknown serum was mixed with one drop of reagent of latex particles coated with IgG for RF factor. The presence or absence of a visible agglutination indicates the presence or absence of RF in the samples tested.

**Anticcp Test** Accomplished by ELISA method (Eagle Biosciences.)

**Procedure**

1. Hundred μl of calibrators (1 - 5), diluted patient sample and control sera were pipetted to the wells.
2. The plate was cover and incubated for 60 min at room temperature (18 - 25 °C).
3. The wells were aspirated sharply striking on filter paper to remove any water droplets residues, wells were washed three times with three hundred micro liters of washing solution.
4. One hundred micro liters of anti-human IgG– horse raddish peroxidase were added to the wells.
5. Microtiter plate were covered, then incubated for thirty minutes at room temperature.
6. The wells were aspirated sharply striking on filter paper to remove any water droplets residues, and then wells were washed three times with three hundred micro liters of washing solution.
7. One hundred microliters of the solution of substrate were added to the wells and were shaken for seconds.
8. The wells were incubated in dark place for fifteen minutes in room temperature.
9. One hundred microliters of stop solution were added to the wells.
10. After thirty minutes, the absorbance was read at 450 nm.

**Calculations** was accomplished using standard curve by plot the absorbance of calibrators from one to five on the y-axis and the concentration of the calibrators of CCP-Ab on the x-axis as shown in table (1). The CCP Ab calculated in U/ml.

<table>
<thead>
<tr>
<th>No. of calibrators</th>
<th>Concentrations in U/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
</tr>
<tr>
<td>5</td>
<td>2000</td>
</tr>
</tbody>
</table>

**Figure 1** Standard curve, no. of calibrators on x-axis and their absorbance on y-axis
Cut Off: Negative < 30 U/ml, Positive ≥ 30 U/ml

**Results**

Figure (1) shows all patients in this study according to sex. It was clear that women were more than men with PsA (figure 3), while males were more than females with cutaneous psoriasis (figure 2). The present study found that 30 (36.58%) out of 82 had PsA while 52 (63.41%) had psoriasis.

Mean age of all patients was 48 years, mean age of women was 39 years and for men was 42 years. Mean age of PsA patients was 35 years mean age of women was 33 years and for men was 35 year. Mean age of patients with cutaneous psoriasis was 41 years, while mean age of men was 44 years and for women was 41 years. Anti CCP was positive in only one patient with cutaneous psoriasis (2%) while 10 with PsA had positive anti CCP (33.33%), 7 of them were followed up for 1 year and it was found that there is an increase in the levels of anticcp (range 34 U/ml-1500 U/ml).

RF was positive in only 5 (16.7%) cases with PsA and all 5 (50%) had a positive anticcp, whereas no patients with cutaneous psoriasis were RF positive.

Control persons had normal values in all above tests.

As shown in the figure 4 there is an increasing concentration of anticcp during the 4 trimesters especially in the 3rd and 4th trimesters of the pts number 6 and 7, while there is a great increase in anticcp concentration in the 4th trimester of pt number 5 in comparison to 3rd trimester.

**Figure 1** No. of all pts. according to sex
Table 1 Parameters and no. of pts. of the groups

<table>
<thead>
<tr>
<th>Disease</th>
<th>PsA</th>
<th>Psoriasis</th>
<th>PsA&amp;Psoriasis</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>30</td>
<td>52</td>
<td>82</td>
<td>14</td>
</tr>
<tr>
<td>Age(yrs)</td>
<td>35</td>
<td>41</td>
<td>48</td>
<td>39</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>22</td>
<td>42</td>
<td>8</td>
</tr>
<tr>
<td>Male</td>
<td>10</td>
<td>30</td>
<td>40</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 2 Tests performed for groups

<table>
<thead>
<tr>
<th>Disease</th>
<th>PsA</th>
<th>Psoriasis</th>
<th>PsA&amp;Psoriasis</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR(mm/hr.)</td>
<td>36</td>
<td>82</td>
<td>60</td>
<td>5</td>
</tr>
<tr>
<td>CRP(+ve)</td>
<td>28</td>
<td>17</td>
<td>45</td>
<td>4</td>
</tr>
<tr>
<td>RF</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Anticcp</td>
<td>1</td>
<td>10</td>
<td>11</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3 Anticcp concentration in U/ml follow-up of 7 patients with PsA

<table>
<thead>
<tr>
<th>No.pts</th>
<th>1st Trimester</th>
<th>2nd Trimester</th>
<th>3rd Trimester</th>
<th>4th Trimester</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>47</td>
<td>71</td>
<td>200</td>
</tr>
<tr>
<td>2</td>
<td>36</td>
<td>51</td>
<td>105</td>
<td>203</td>
</tr>
<tr>
<td>3</td>
<td>39</td>
<td>68</td>
<td>73</td>
<td>85</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>83</td>
<td>214</td>
<td>800</td>
</tr>
<tr>
<td>5</td>
<td>51</td>
<td>70</td>
<td>83</td>
<td>1000</td>
</tr>
<tr>
<td>6</td>
<td>57</td>
<td>93</td>
<td>866</td>
<td>1500</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>211</td>
<td>790</td>
<td>1500</td>
</tr>
</tbody>
</table>

As shown in the Figure 4 there is an increasing concentration of anticcp during the 4 trimesters especially in the 3rd and 4th trimesters of the patients number 6 and 7, while there is a great increase in anticcp concentration in the 4th trimesters.
trimester of patient number 5 in comparison to 3rd trimester.

**Discussion**

The results shown in Figures 1-3 are in accordance with the study by Candi et al. who stated that 47% of psoriatic patients have PsA. 42 (51.21%) were female and 40 (48.78%) were male, 20 (66.66%) out of 30 patients with PsA were female and 10 (33.33%) were male, 22 (42.3%) out of 52 patients of cutaneous psoriasis were female and 30 (57.7%) of them were male. Popescu et al. stated mean age 50.2 years for patients with PsA. Others found that age range of psoriasis was (23-42) years and mean age of PsA was (35-50) years. According to Table 2 a study on patients with immune skin disorders Kumari and colleagues stated that anticcp of a significant association in those patients with arthritis. In comparison to the present study Pay et al. confirmed that 10.6% of PsA had positive anticcp, results of Candi and coworkers was far from ours they found a percent of 9.72%, and Ohashi et al. found 7.9% in patients with pustuloticarthritis (PAO); another study by Cheng et al. in 2018 stated that anticcp is could be of greater in patients with fracture, while another author stated that anticcp antibody produces an inflammatory symptom in RA patients. And about RF other workers found that 7% and 0.7% of patients with PsA and psoriasis respectively had positive results of anticcp. This weak association goes with Candi et al., 2006; Popescu et al, 2013; and Korendowycz et al., 2005 and in comparison, with Alenius et al., 2006 and Di Minno et al, 2012. While the result of CRP goes with Strober et al. in 2008, Punzi and coworkers found that ESR and CRP are not very important in diagnosis of PsA because their levels increased in only 50% of patients, the same researchers stated that 5% to 13% of PsA patients had positive results of RF and anticcp, others had reach to another results, 2% of psoriatic patients (ages > 40) years had positive results of RF all of them had previous history of RA. With regard to age, sex, CRP and ESR, the present study doesn’t find any differences between patients having positive anticcp and patients with negative anticcp. Punzi and colleagues concluded that no lab test diagnose PsA, RF could distinguish PsA in some cases from RA, while anticcp have an important role in this field.

Also, about Table 3 Popescu et al. found 12.2% of patients were positive for anticcp. Candi and colleagues stated the usefulness of both anticcp and RF in the distinguishing between RA and PsA, while Popescu and coworkers stated that there is an association between anticcp antibodies and features of PsA and also proved the importance of those autoantibodies in distinguishing patients with PsA who have symptoms just like arthritis from others with RA.

Referring to the Table 4 (that contain 7 patients under follow up) these results help us to conclude that anticcp is a good indicator for follow up study of PsA pts.

**References**

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