A Comparative Study of Serum Amyloid A2 with Anti-cyclic Citrullinated Peptide antibody in the prognosis of a Group of Rheumatoid Arthritis Patients in Iraq

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Mohammed H. Alosami *** CABM, FIBMS

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Abstract:

Background: Rheumatoid arthritis (RA) is an inflammatory disease of unknown etiology characterized by joint inflammation and the presence of autoantibodies, mostly Anti-cyclic citrullinated peptide antibody (ACCP) which are released when the body loses its ability to distinguish between self and foreign molecules. Serum amyloid A2 (SAA2) is an acute phase protein produced in response to inflammatory conditions including RA.

Objectives: To investigate the prognostic ability of SAA2 in comparison with ACCP and the prediction of disease activity and response to treatment by Methotrexate and Etanercept in Iraqi RA patients.

Patients and methods: A case control study, on a total of 150 individuals; 100 patients and 50 healthy controls. The study was carried out between November 2021 to February 2022 in Baghdad Teaching Hospital. The patients were recruited according to the American College of Rheumatology 2010 criteria. The biomarkers’ levels were measured by enzyme-linked immunosorbent assay.

Results: The levels of ACCP and SAA2 in RA patients were significantly higher compared to healthy controls (p<0.001), and were higher among patients with active disease than those with inactive disease, with the levels being higher in both categories than the healthy controls (p<0.00). Highly significant ACCP levels were found in patients without treatment than those who have received treatment (methotrexate or etanercept) (p<0.00). SAA2 level in patients without treatment were not significantly different from those of patients who have received methotrexate (p>0.05) but significantly from those who have received etanercept (p<0.02). A significant positive correlation was found between ACCP and SAA2 (r=0.53, p<0.001), with the sensitivity being (72%, 97%) and the specificity being (98%, 84%) respectively.

Conclusion: ACCP and SAA2 have promising prognostic ability and disease activity prediction of RA with response to treatment (Methotrexate, Etanercept).

Keywords: Rheumatoid arthritis, Anti-cyclic Citrullinated peptide antibody, Serum amyloid A2, Methotrexate, Etanercept.

Introduction:

Rheumatoid arthritis (RA) is an inflammatory autoimmune chronic condition where immune cells interact with soluble mediators. It primary affects the small joints, with the extra-articular manifestations involving the skin, nerves, and eyes as well as the gastrointestinal, cardiovascular, pulmonary and renal systems (1, 2, 3, 4). It is a disease of unknown etiology (5). The worldwide prevalence of RA being 0.5-1.0%, females are 2 - 3 times more frequently affected than males and the ages between 30 - 50 years are at a higher risk of developing RA (6, 7). The pathogenesis of RA is through the auto-reactive B cells which contribute to auto-antibodies production and T cells by release of cytokines after activation (8). Antibodies against citrullinated peptides including IgG, IgM, and IgA isotypes which are known as Anti-cyclic Citrullinated peptide antibodies (ACCP), and are associated with joint destruction and increase the risk of disease progression (9). The first line of the treatment strategy is disease-modifying anti-rheumatic drugs (DMARDs) which reduce rheumatoid arthritis aggressiveness. If a patient does not respond to synthetic DMARDs, biological therapy should be initiated which has different targets such as Interleukin – 6 (IL-6) and Tumor necrosis factor-alpha (TNF-a) (10, 11). Serum amyloid A (SAA) is an acute phase protein and marker for a variety of disorders including infections, inflammatory...
reactions and malignancies. SAA levels rise from 10- to 100-fold during inflammation and reach up to 1000-fold after severe inflammatory conditions (12). SAA plays a significant role in inflammatory rheumatic disorders (IRD), such as its involvement in the activation of the inflammatory cascade and the recruitment of interleukin-17 producing T helper cells. SAA has an essential role in the pathophysiology of (IRD) (13). Overproduction of SAA in RA because of its continuous release and the significant acute-phase response leading to the development of amyloidosis which is a chronic condition a resulting from the continuous release of SAA including SAA2 (14). Inflammatory cytokines such as Interleukins (IL-1, IL-6) and tumor necrosis factor–alpha (TNF-α) can activate the production of SAA1 and SAA2, which are highly expressed in the liver and are elevated during the acute phase response so they are known as acute-SAA proteins (A-SAA) (12).

Patients and Methods:

Study design: A Case-control study was conducted in Baghdad Teaching Hospital \ Rheumatology Consultation Clinic between November 2021 to February 2022.

Inclusion criteria: RA patients diagnosed according to the American College of Rheumatology 2010 criteria, grouped into those who have received DMARDs (Methotrexate 2.5 mg), or Biological DMARDs (Etanercept 50 mg) and those without treatment.

Exclusion criteria: Those with an active infection, rheumatologic autoimmune diseases other than RA, or systemic disorders and RA patients who have received (DMARDs) treatment other than methotrexate, patients who have received methotrexate (MTX) dose > 2.5 mg, and patients who have received biological (DMARDs) other than Etanercept 50 mg.

Patients' subgroups:

The study included the following groups:

1) The patients group: Included (100) RA patients (83 females and 17 males) with an age range of (19-65) years who were diagnosed by a specialist rheumatologist through clinical examination confirmed by laboratory investigations and radiological imaging.

This group was further subdivided into 3 subgroups:

A) First sub-group (37 Patients) who have taken DMARDs (MTX 2.5 mg).
B) Second sub-group (42 Patients) taking bDMARDs (etanercept 50mg).
C) Third sub-group (21 patients) without treatment for 2 months or more.

2) Healthy control group: It included 50 apparently healthy persons (43 females and 7 males) with an age range between (22-72) years who were matched with ages and genders with patients' group.

Blood samples collection: Five milliliters of venous blood samples were collected from patients and controls.

Serum separation: The serum which was obtained from the blood samples was left for 30 minutes at room temperature, then centrifuged at 3000 rpm for ten minutes. The serum for each sample was collected in eppendorf tubes and stored at -20°C until the time it was used for testing by enzyme-linked immunosorbent assay (ELISA), for measuring ACCP (Aeskulisa- Germany), SAA2 (Sunlong-Biotech-China).

Data Collection: 1- Name, age, disease duration, gender, family history, fulfilling the American College of Rheumatology criteria 2010 (ACR-2010), 2- Assessment of clinical disease activity index (CDAI) by clinical examination.

3- History of the disease and types of medication taken currently and previously.

4- Laboratory investigations: including erythrocyte sedimentation rate (ESR) and complete blood picture (CBC).

Statistical analysis: The Statistical Package for Social Sciences (SPSS) version 26 and (Microsoft Excel 2010) were used for statistical analysis of the study data including mean, SD, range, independent T-test for determination of statically significant differences between group means. One way ANOVA test to determine statistical differences between subgroups. Statistical significance was determined when p<0.05, and p<0.001 indicated high significance. Pearson correlation and regression analysis were used to determine the correlation coefficient between study variables. The receiver operating characteristic curve (ROC curve) was used to determine cut-off points, sensitivity and specificity of tests.

Results:

The patients were sub-divided into four age groups ≤ 35 years (18%), 36 – 45 years (22%), 46 – 55 (35%) and ≥ 56 years (25%). There were 87% smokers and 13% non-smokers among the patients. The mean disease duration was 9.1 ± 8.25 years with a range of 6 months - 40 years. Family history and signs and symptoms such as fever, redness, tender joints, swollen joints, Visual analogue scale, demographic and clinical characteristics and related features of RA illness are shown in Table (1).

Table 1: Family history and clinical characteristics of RA patients

<table>
<thead>
<tr>
<th>RA variables in 100 RA patients</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history of RA</td>
<td>18 (18)</td>
</tr>
<tr>
<td>Fever</td>
<td>64 (64)</td>
</tr>
<tr>
<td>Redness</td>
<td>37 (37)</td>
</tr>
<tr>
<td>Tender joints mean ± SD</td>
<td>8.9 ± 5.53</td>
</tr>
<tr>
<td>Swollen joints mean ± SD</td>
<td>1.1 ± 1.13</td>
</tr>
<tr>
<td>Visual Analogue Scale (0-10) mean ± SD</td>
<td>5.4 ± 1.73</td>
</tr>
</tbody>
</table>

The clinical disease activity index (CDAI) of RA patients included in the study was 20.3 ± 8.89 with a range of (1-45). Twelve RA patients had low disease activity (mean±SD CDAI 5.9±3.20, range 1- 9), 46 had moderate disease activity (mean±SD
CDAI 16.6±3.84, range 10- 22), and 42 had high disease activity (mean±SD CDAI was 28.6±5.11, range 23 - 45), p<0.001, Figure (1).

Figure 1: Means of CDAI in RA patients

Tass for the medications received by the RA patients, 37 patients received DMARDs (MTX), 42 patients received biological DMARDs (etanercept) and 21 patients were without any treatment for two months or more. Lab investigations of RA patients and their healthy controls including ESR, ACCP and SAA2 show highly significant differences (p<0.001), Table (2).

Table 2: Results of lab investigations of RA patients and healthy controls

<table>
<thead>
<tr>
<th>Investigations</th>
<th>Groups</th>
<th>Mean ± SD</th>
<th>P – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR (mm / hr)</td>
<td>RA patients</td>
<td>39.9 ± 25.47</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>11.6 ± 8.9</td>
<td></td>
</tr>
<tr>
<td>ACCP (U/ml)</td>
<td>RA patients</td>
<td>59.0 ± 13.04</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>5.1 ± 1.70</td>
<td></td>
</tr>
<tr>
<td>SAA2 (μg/L)</td>
<td>RA patients</td>
<td>25.6 ± 3.99</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>12.3 ± 4.73</td>
<td></td>
</tr>
</tbody>
</table>

ESR: Erythrocytes sedimentation rate, ACCP: Anti-cyclic Citrullinated protein/peptide antibody, SAA2: Serum amyloid A2, SD: Standard deviation

ACCP and SAA2 levels in RA patients to the activity of the disease based on CDAI show that levels in patients with active disease were significantly higher than those with inactive disease (moderate and low disease activity). All three activity categories levels were higher than healthy control level, Table (3).

Table 3: Mean ± SD of biomarkers in controls and RA patients grouped according to CDAI levels

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Healthy Control N=50</th>
<th>RA Patient groups according to CDAI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No=100</td>
<td>Low disease activity CDAI ≤ 9 Moderate disease activity CDAI ≤ 22 High disease activity CDAI &gt; 22</td>
<td></td>
</tr>
<tr>
<td>ACCP (U/ml)</td>
<td>5.1±1.70</td>
<td>20.5±5.12</td>
<td>38.3±18.79</td>
</tr>
<tr>
<td>SAA2 (μg/L)</td>
<td>12.3±4.73</td>
<td>20.0±1.15</td>
<td>24.9±2.61</td>
</tr>
</tbody>
</table>

ACCP: Anti-cyclic Citrullinated peptide antibody, SAA2: Serum amyloid A2, SD: Standard deviation, CDAI: Clinical disease activity index

The level of ACCP in patients without treatment were significantly higher than in patients who have received treatment (Methotrexate or etanercept) (p<0.001). The difference between the ACCP levels among patients who have received MTX and patients who have received etanercept was not statistically significant (p>0.05). Moreover, the SAA2 level of patients without treatment was not statistically different from that of patients who have received MTX (p>0.05), but it was significantly higher than that of patients who have received etanercept (p<0.02). The difference between the SAA2 level of patients who have received MTX and patients who have received etanercept was not statistically significant (p>0.05), Table (4).
The Correlation between the biomarkers and the study variables was a significant positive correlation between ACCP and ESR ($r=0.258$, $p<0.01$), ACCP and disease activity (CDAI) ($r=0.459$, $p<0.001$), and ACCP and SAA2 ($r=0.203$, $p<0.05$), SAA2 and CDAI ($r=0.544$, $p<0.001$) and SAA2 and ACCP ($r=0.553$, $p<0.001$) Figure (2).

Figure 2: Scatter diagram showing the correlation between ACCP and SAA2 in RA patients

The receiver operating characteristic curve (ROC curve) was utilized for comparison between ACCP and SAA2 for RA detection and evaluation of sensitivity, specificity and cutoff values. The area under curve (AUC) of ACCP was (0.87) cutoff value ($>13.13$ U/ml) Sensitivity (72%), Specificity (98%), (AUC) for SAA2 was (0.92) cutoff value $>19.57$ μg/L Sensitivity (97%), Specificity (84 %) Figure (3).

Figure 3: ROC curve of ACCP and SAA2

Discussion:
The female to male ratio among the RA patients in the current study was 4.8:1, which may be due to the effect of environmental and hormonal factors on the RA inflammatory reactions and the production of cytokines in the synovium which can directly affect the cartilage. This female preponderance is in accordance with local and international studies (15,16). About one third of the RA patients fell in the age group of (46–55 years), which was higher than the three other age groups. Older age is associated with a decrease of humoral immunity and immune defense mechanism leading to an increased predisposition to autoimmune conditions. This result agrees with local and international studies which have shown that the age of RA patients was over 40 years (17, 18). One of the main age-related alterations in the compartments of B cell is the accumulation of auto-reactive B lymphocytes which is known to be age-associated, with a variation in B cells receptor and gene expression of human leucocytes antigens HLA-DR1 and HLA-DR4 susceptible genes (19, 20). RA is exaggerated by the inflammatory conditions and ESR is non-specific laboratory marker the level of which increases during the inflammatory process. Our results agree with a local study which has shown that ESR level in RA patients was significantly higher in comparison healthy controls (21). The levels of ACCP and SAA2 in RA patients’ serum were significantly higher than healthy control in accordance with other studies (22, 23). The levels of SAA2 are persistently raised in a variety of T helper 17-mediated autoimmune disorders including RA (24). The elevation of ACCP levels in active RA patients may be due to increasing citrullination and inflammation which also increase immune complex formation with the recruitment of a large number of immune cells after complement activation. Our results agree with those of a study which showed that the serum ACCP level in RA patients is increased with increasing severity of the disease and that higher ACCP levels in severe and moderate disease activity in comparison with mild activity, but ACCP levels in all disease stages were higher than in healthy controls (25). SAA2 is considered a major acute phase protein synthesized in the liver in response to inflammatory conditions including acute active inflammatory synovitis. Another study had shown similar results that significantly higher levels of SAA2 were detected in patients with active RA disease in comparison with healthy controls but they were not significantly different among mild and moderate severity of the disease (23). The reason for ACCP being higher among those without treatment than those with treatment is due to the immunosuppressant effect of MTX and the anti-cytokine activity of etanercept. Another study suggested that this level was significantly decreased in the serum of patients who have received etanercept and is lower than RA patients without treatments (26). SAA2 as well as

![Table 4: Mean ± SD of biomarkers according to treatment](image)

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Without treatments</th>
<th>With treatments</th>
<th>P – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACCP (U/ml)</td>
<td>82.8 ± 55.3</td>
<td>55.0 ± 31.7</td>
<td>51.5 ± 11.63</td>
</tr>
<tr>
<td>SAA2 (μg/L)</td>
<td>27.2 ± 6.45</td>
<td>25.8 ± 3.57</td>
<td>24.6 ± 3.80</td>
</tr>
</tbody>
</table>

SAAs are isoforms of the SAA family and are related to the acute phase proteins (23). The correlation between SAA2 and CDAI was a significantly positive correlation. A previous study indicated a direct correlation between SAA2 and disease activity (23). The significantly positive correlation between SAA2 and ACCP was due to high SAA2 levels in seropositive which are associated with higher levels of ACCP patients, ACCP positive in comparison with seronegative patients (ACCP negative), in line with another study which found a significant positive correlation between ACCP and serum amyloid (SAA2) (28).

Accordingly, SAA2 is an important biomarker for RA which can be used like other preclinical biomarkers in RA such as C-reactive protein (CRP), Rheumatoid Factor (RF) and ACCP (23). SAA is a more sensitive biomarker than ESR and CRP (13). Another study which studies the sensitivity and specificity of ACCP found that they were (67%, 85-95%) respectively (29), while another study found the sensitivity of SAA2 to be more than 50% and the specificity to be more than 80% (30).

Conclusions:
Anti-citrullinated peptide antibody in addition to SAA2 have promising prognostic ability and to differentiation between RA patients and healthy controls and can predict the severity of the disease in response to treatment (methotrexate, etanercept). Significant positive correlation between ACCP with SAA2 can enhance the prediction of RA prognosis.

Authors’ contributions:
Ali A.Oglah, Khalil I. Abid and Mohammed H. Alosami were contributor on the study concept and design, Ali A.Oglah and Khalil Ismail Abid were contributor on samples, data collections analysis, the manuscript drafting and interpretations, Critical review was by Mohammed Hadi. Alosami.

References:
دراسة مقارنة أالأمليود المصلي A2 مع الأجسام المضادة للببتيد السترويليني الحلقي في تكه مرض
الالتهاب المفاصل الروماتويدي عند مجموعة من المرضى في العراق

د. علي عيدالإ>Description

أ.د. خليل مباشر عبد محمد
أ.د. محمد هادي العصامي

الخليصة: تكه المفاصل الروماتويدي هو مرض إلتهابي مجهول السبب مرتبط بالالتهاب المفاصل ووجود أجسام مضادة ذاتية، معظمها أجسام مضادة للكلا النشاطين. كان مستوى الأميلويد A2 في أحاد إحصائية III مرضى مصابين بالالتهاب المفاصل الروماتويدي كان أعلى من المرضى الذين لم يتلقوا علاجات مرض الطريق (الميتوتركسيت أو إيتانيرسبت). كان مستوى الأميلويد المصلي A2 في المرضى الذين لم يتلقوا علاجات غير ذو دالة إحصائية مع الفحص عند الفحص الأديل بين الأجسام المضادة للكلا النشاطين. كان متوسط الأجسام المضادة للببتيد السترويليني الحلقي في جميع المرضى كان أعلى من المرضى الذين لم يتلقوا علاجات غير ذو دالة إحصائية مع الفحص عند الفحص الأديل بين الأجسام المضادة للكلا النشاطين. كان متوسط الأجسام المضادة للببتيد السترويليني الحلقي في جميع المرضى كان أعلى من المرضى الذين لم يتلقوا علاجات غير ذو دالة إحصائية مع الفحص عند الفحص الأديل بين الأجسام المضادة للكلا النشاطين. كان متوسط الأجسام المضادة للببتيد السترو일يني الحلقي في جميع المرضى كان أعلى من المرضى الذين لم يتلقوا علاجات غير ذو دالة إحصائية مع الفحص عند الفحص الأديل بين الأجسام المضادة للكلا النشاطين. كان متوسط الأجسام المضادة للببتيد السترويليني الحلقي في جميع المرضى كان أعلى من المرضى الذين لم يتلقوا علاجات غير ذو دالة إحصائية مع الفحص عند الفحص الأديل بين الأجسام المضادة للكلا النشاطين. كان متوسط الأجسام المضادة للببتيد السترويليني الحلقي في جميع المرضى كان أعلى من المرضى الذين لم يتلقوا علاجات غير ذو دالة إحصائية مع الفحص عند الفحص الأديل بين الأجسام المضادة للكلا النشاطين. كان متوسط الأجسام المضادة للببتيد السترويليني الحلقي في جميع المرضى كان أعلى من المرضى الذين لم يتلقوا علاجات غير ذو دالة إحصائية مع الفحص عند الفحص الأديل بين الأجسام المضادة للكلا النشاطين. كان متوسط الأجسام المضادة للببتيد السترويليني الحلقي في جميع المرضى كان أعلى من المرضى الذين لم يتلقوا علاجات غير ذو دالة إحصائية مع الفحص عند الفحص الأديل بين الأجسام المضادة للكلا النشاطين. كان متوسط الأجسام المضادة للببتيد السترويليني الحلقي في جميع المرضى كان أعلى من المرضى الذين لم يتلقوا علاجات غير ذو دالة إحصائية مع الفحص عند الفحص الأديل بين الأجسام المضادة للكلا النشاطين. كان متوسط الأجسام المضادة للببتيد السترويليني الحلقي في جميع المرضى كان أعلى من المرضى الذين لم يتلقوا علاجات غير ذو دالة إحصائية مع الفحص عند الفحص الأديل بين الأجسام المضادة للكلا النشاطين. كان متوسط الأجسام المضادة للببتيد السترويليني الحلقي في جميع المرضى كان أعلى من المرضى الذين لم يتلقوا علاجات غير ذو دالة إحصائية مع الفحص عند الفحص الأديل بين الأجسام المضادة للكلا النشاطين. كان متوسط الأجسام المضادة للببتيد السترويليني الحلقي في جميع المرضى كان أعلى من المرضى الذين لم يتلقوا علاجات غير ذو دالة إحصائية مع الفحص عند الفحص الأديل بين الأجسام المضادة للكلا النشاطين. كان متوسط الأجسام المضادة للببتيد السترويليني الحلقي في جميع المرضى كان أعلى من المرضى الذين لم يتلقوا علاجات غير ذو دالة إحصائية مع الفحص عند الفحص الأديل بين الأجسام المضادة للكلا النشاطين. كان متوسط الأجسام المضادة للببتيد السترويليني الحلقي في جميع المرضى كان أعلى من المرضى الذين لم يتلقوا علاجات غير ذو دالة إحصائية مع الفحص عند الفحص الأديل بين الأجسام المضادة للكلا النشاطين. كان متوسط الأجسام المضادة للببتيد السترويليني الحلقي في جميع المرضى كان أعلى من المرضى الذين لم يتلقوا علاجات غير ذو دالة إحصائية مع الفحص عند الفحص الأديل بين الأجسام المضادة للكلا النشاطين. كان متوسط الأجسام المضادة للببتيد السترويليني الحلقي في جميع المرضى كان أعلى من المرضى الذين لم يتلقوا علاجات غير ذو دالة إحصائية مع الفحص عند الفحص الأديل بين الأجسام المضادة للكلا النشاطين. كان متوسط الأجسام المضادة للببتيد السترويليني الحلقي في جميع المرضى كان أعلى من المرضى الذين لم يتلقوا علاجات غير ذو دالة إحصائية مع الفحص عند الفحص الأديل بين الأجسام المضادة للكلا النشاطين. كان متوسط الأجسام المضادة للببتيد السترويليني الحلقي في جميع المرضى كان أعلى من المرضى الذين لم يتلقوا علاجات غير ذو دالة إحصائية مع الفحص عند الفحص الأديل بين الأجسام المضادة للكلا النشاطين. كان متوسط الأجسام المضادة للببتيد السترويليني الحلقي في جميع المرضى كان أعلى من المرضى الذين لم يتلقوا علاجات غير ذو دالة إحصائية مع الفحص عند الفحص الأديل بين الأجسام المضادة للكلا النشاطين. كان متوسط الأجسام المضادة للببتيد السترويليني الحلقي في جميع المرضى كان أعلى من المرضى الذين لم يتلقوا علاجات غير ذو دالة إحصائية مع الفحص '_'})

جملة مفتاحية: تكه المفاصل الروماتويدي، الأجسام المضادة للببتيد السترويليني الحلقي، الأميلود المصلي A2، ميتوتركسيت، إيتايرسبت.