Evaluation of serum Interleukin 36 in Iraqi patients with Rheumatoid arthritis

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Abstract: Rheumatoid arthritis is a worldwide inflammatory chronic autoimmune disease with varying severity. Due to no definitive cure for this disease, current therapies aim to decrease the pain and slow further damage. The interleukin (IL)-36 cytokine was little known for its role in rheumatoid arthritis; this research aimed to evaluate the serum IL36 levels in RA patients compared to healthy controls. This study included 80 patients with rheumatoid arthritis registered at the Rheumatology Clinic in Baghdad teaching hospital. The patients were divided into three groups based on the treatments received. Group 1 included patients treated with biological therapy (etanercept, adalimumab), Group 2 patients with non-biological treatment (methotrexate hydroxychloroquine and prednisone), Group 3 patients without any treatment and compared with Group 4 healthy control group. Patients in all groups were assessed for their serum IL-36 concentration; the mean IL-36 serum level was significantly higher in three groups of RA patients which include the group of patients treated with biological therapy (Enbrel (etanercept) and Humira (adalimumab)) means were (1132.41±475.2,), and group of non-biological therapy patients (Methotrexate hydroxychloroquine and prednisone) (G2) means was 553.95±307, than patients' group without any treatment (G3) means was 1044.01±575.3 compared to the control (341.38±113.1) p-value>0.00001. The patient's age and BMI were not significantly different between three groups of patient Rheumatoid arthritis. Parameters for this disease also were tested which include RF, CRP, ESR, anti-CCP and disease activity score-28 (DAS 28), there were significant differences when compared with the control group. IL-36 serum level was significantly higher in three groups of rheumatoid arthritis than those in controls, and when compared between three patients groups there was less concentration in the non-biological therapy treatment group means was 553.95±307 than in the rest of the patient groups, biology tratment, without any treatment, means were (1132.41±475.2, 1044.01±575.3) respectively. This study found that Rheumatoid arthritis patients' serum IL36 levels increased, where a non-biologic therapies reduced this cytokine. IL-36's pathogenic involvement in Rheumatoid arthritis needs more study.

Key words: Rheumatoid arthritis, IL-36, IL-1, C-reactive protein, RF, ESR and anti-CCP.

Introduction

Rheumatoid arthritis (RA) is a worldwide inflammatory chronic autoimmune disease with varying severity, and due to no definitive cure for this disease, the goals of current therapies are to decrease the pain and slow further damage. Rheumatoid arthritis affects small joints after that, larger joints, finally leading the tendons and ligaments weaken, and the cartilage and bone of joints are distortion and erosion which cause severe pain for a patient, and affects on heart, kidneys, lungs skin, and eyes. Commonly autoimmune diseases are characterized by an excessive immune response and cause deterioration of specific or multiple tissues and organs, and generally believed that cytokines implicated in each phase of the pathogenesis of RA, such as IL-1β, IL-6, IL-8, IL-10, IFN-gamma, etc. Interleukin 36 is an inflammatory cytokine, a member of the IL1 family, composed of agonists IL36α, IL36β, IL36γ, and IL-36Ra antagonist IL-36 receptor IL36Ra and accessory protein (IL-1RaCP)⁸, chromosome 2 carries the genes of the IL-36 family, expressed and act on the barrier sites of the body on a variety of cells including epithelial, synoviocytes, keratinocytes, and skin, lung, and gut cells and immune cells (T lymphocytes, antigen-presenting cells). IL-36 cytokines are regulated immune responses in a specific tissue; the upregulation of IL36 through CD80, CD86, MHCII of dendritic cells induces the production of several proinflammatory cytokines such as IL-1β, IL-6, IL-8, IL-17, and TNFα and IFNγ in the pathogenesis of inflammatory diseases, in lung tissue, joint synovium (arthritis), colonic mucosa tissues (bowel diseases) and skin lesion and may have diagnostic and/or therapeutic relations with inflammatory diseases. In RA patients, IL-36α, IL-36R, and IL-36Ra were detected in the synovial tissues and may be correlated with its pathogenesis. In the synovium of RA patients, IL-36α, IL-36β, IL-36γ, and IL-36Ra are correlated and upregulated with the expression of IL-1β, Chemokine ligand 3, Chemokine ligands 4 and Macrophage colony-stimulating factor, detected the IL-36 cytokines in the synovium of RA patients which induce production of proinflammatory mediators by synovial fibroblasts. Still, there were no effects when the blockade of IL-36 receptors in the arthritis of mouse models the production of proinflammatory mediators by synovial fibroblasts. Found the circulating IL-36 levels were significantly higher among Juvenile Idiopathic Arthritis children. The present study examined the serum concentration of IL-36 in rheumatoid arthritis patients.

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**Materials and methods**

All serum samples of 80 rheumatoid arthritis patients (11 males and 69 females) who attended the Rheumatology Clinic in Baghdad teaching hospital and diagnosis was under the supervision of Dr. Muhammad Hadi Al-Assami, Consultant Rheumatologist, according to the revised American College of Rheumatology 2010 criteria, based on 4 factors which are the distribution of affected joints number tender joint and swollen, Serology test results rheumatoid factor (RF), anti-cyclic citrullinated peptide antibodies (anti-CCP) kit, and it was calculated using the following equation:

\[ \text{DAS28} = 0.56 \times \sqrt{\text{tender 28 joint count}} + 0.28 \times \sqrt{\text{swollen 28 joint counts}} + 0.70 \times \ln{\text{erythrocyte sedimentation rate (ESR), mm/hr}} + 0.014 \times \text{general health} \]

Clinicians used criteria to diagnose rheumatoid arthritis. Presence of at least one clinical tumor in the participant), up to three points are assigned depending; it was done by the consultant medical staff at the clinic, in addition to using the kits for laboratory tests to confirm the presence of arthritis such as anti-cyclic citrullinated peptide antibodies (anti-CCP) kit (commercially available kits by indirect enzyme-linked immunosorbent assay MyBioSource, USA), rheumatoid factor (RF) and C-reactive protein (CRP) kits (latex slide agglutination tests by Agappe Diagnostics Switzerland GmbH for semi-quantitative detection). The selection of patients, according to what was mentioned in the materials and methods, from patients who visit a specialized center for rheumatic diseases and who were diagnosed according to the revised American College of Rheumatology 2010 criteria by specialists and the general parameters for this disease also were tested which include RF, CRP, ESR, anti-CCP and disease activity score-28 (DAS 28), and there were significant differences when compared with the control group because the latter was chosen with great caution. It excluded anyone who gave a positive result to any of those above to confirm that the control group members were free from RA.

The demographic and clinical features of the 80 patients with rheumatoid arthritis included in this study are displayed in Table 1 according to the above-mentioned division in the materials and methods. There were no significant differences observed in the age at sample collection between the patients with RA and the healthy control group (P > .05). The mean age of the three patient groups: treated with the biological treatment group was 43.8±2.3, the non-biological treatment group was 46.1±2.2, and the non-treatment group was 40.3±3.2 and healthy controls with a mean age of 46.7±2.9 years (Table 1).

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<th>Variables</th>
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<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>P value</th>
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<td>Age(Mean±SD)</td>
<td>3.8±2.3</td>
<td>46.1±2.2</td>
<td>40.3±3.2</td>
<td>46.7±2.9</td>
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<tr>
<td>Total patient</td>
<td>38</td>
<td>26</td>
<td>16</td>
<td>36</td>
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<tr>
<td>Female</td>
<td>31</td>
<td>24</td>
<td>14</td>
<td>18</td>
<td>0.0008</td>
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<tr>
<td>Male</td>
<td>7</td>
<td>2</td>
<td>2</td>
<td>18</td>
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<tr>
<td>Sex M/F</td>
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<td>12/1</td>
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**Table 1.** Demographic characteristics of RA patients and healthy controls.
Discussion

There were significant differences between patients with rheumatoid arthritis and normal controls in terms of sex (Table I); the ratio of female to male was 6:1 in this study, and in general, according to previous studies, women are about three times more likely to suffer from this disease, and the effect of the disease is also different between the two sexes, and this may be due to physiological differences between the sexes, such as the difference in hormonal content, the difference in behavior, and the role of genes and heredity. RA can be triggered by the production of autoantibodies (anti-citrullinated protein antibodies ACPA) against citrullinated peptides which are distributed throughout the whole body that activate MHC class II-dependent T cells that induce B cells to produce more ACPA. The mean IL-36 serum level was significantly higher in three groups of RA patients, these results are in line with a previous study, soon published, where it was found that interleukin-36 increases in patients with Juvenile Idiopathic Arthritis. High levels of interleukin-36 were observed in patients and mouse models with osteoarthritis through joint destruction and found that transforming growth factor-beta (TGF-β) receptor type 2 signaling dampened IL-36 signaling in healthy joints, found that IL-36 upregulated in the synovial tissue of patients with RA and they added that particularly linked to the inflammatory processes in the synovial tissue through promotes the expression of proinflammatory cytokines, this confirmed by found that the serum IL-36 higher in SLE patients with arthritis than in those without arthritis. IL-36 acts as a proinflammatory by magnifying inflammatory responses and triggering further inflammatory mediators and causing excessive immune infiltration and tissue damage. Also noted in table 1 interleukin-36 was less concentrated in the non-biological therapy (hydroxychloroquine and prednisone) treatment group (553.95±307) than the rest of the patient groups (1132.41±475, 1044.01±575.3) figure 1, (methotrexate, prednisone) decreases inflammation and suppresses the immune system through binding with specific nuclear receptors and cause altered gene expression and inhibition of proinflammatory cytokine production while hydroxychloroquine treatment prevents trained immunity Many causes depend on the severity of the disease, the stage of the disease, the effect of the treatment and the dose, the physiological factors, the age and this confirmed recently by Devarajan and Vaseghi in their study that hydroxychloroquine impair host immunity in response to SARS-CoV-2 and that demonstrates this results.

According to previous articles, the uses of these two treatments lead to a decrease in the production of inflammatory cytokines; prednisone has a significant effect on inhibiting the immune response through its effect on immune cells and also can reduce the concentration of inflammatory cytokines and hydroxychloroquine is an antimalarial drug

<table>
<thead>
<tr>
<th>Variables</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-CCP</td>
<td>0.34±0.05</td>
<td>0.23±0.05</td>
<td>0.48±0.09</td>
<td>0.12±0.004</td>
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<tr>
<td>ESR</td>
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<td>41.3±3.5</td>
<td>41.1±6.1</td>
<td>15.2±1.2</td>
</tr>
<tr>
<td>DAS 28</td>
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<td>5.06±0.07</td>
<td>4.42±0.13</td>
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</tr>
<tr>
<td>CRP</td>
<td>19</td>
<td>16</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Positive</td>
<td>17</td>
<td>7</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>RF(No.)</td>
<td>17</td>
<td>7</td>
<td>8</td>
<td>0</td>
</tr>
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</table>

Table 2. Laboratory characteristics of the rheumatoid arthritis.
and, is now used as an immunomodulator agent for rheumatic autoimmune disorders, such as primary Sjögren’s syndrome, systemic lupus erythematosus and rheumatoid arthritis through the inhibition of antigen presentation, B- and T-cell activation, NOX signaling and rebalances Treg/Th17 cell ratio and these effects on different immune cells cause decreased in production and release of proinflammatory cytokines\textsuperscript{27,28}.

Conclusions

This study demonstrated that RA patients displayed increasing in serum IL-36 and it was also found that non-biological treatments have a role in reducing this cytokine. Further studies are needed to explain a more detailed IL-36 pathogenic role in RA.

Abbreviations

Rheumatoid arthritis (RA), Anti-Cyclic Citrullinated Peptide antibodies (Anti-CCP), anti-citrullinated protein antibodies (ACPA), Disease active score (DAS 28), Cluster of Differentiation (CD), C-Reactive Protein (CRP), Interleukin (IL), Major Histocompatibility Complex (MHC), Rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), Systemic Lupus Erythematosus (SLE), Intreleukin-1 receptor accessory protein (IL-1RAcP).

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Informed consent to publish

Not applicable.

Ethical statements for human/animal experiments

The study was approved by institutional ethics committee “University of Mustansiriyah” and informed consent was obtained in written by each individual participants. Each participant was known about the study follow up before enrolling for the study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Bibliographic references