

Imbalance between Pro and Anti-Inflammatory Cytokines in Rheumatoid Arthritis in Iraqi Patients

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Abstract:

Background: Rheumatoid arthritis is an autoimmune chronic inflammatory illness that affects the whole body and is characterized by non-articular involvement and inflammatory arthritis. It often develops by an interaction between genes and environmental factors.

Objectives: This research was designed to investigate the effect of the imbalance between pro- and anti-inflammatory cytokines on patients with rheumatoid arthritis and in comparison, with healthy controls.

Methods: Two groups (One hundred) of patients with Rheumatoid arthritis (66female, 34 male) and 50 healthy control group (28female, 22 male)) were chosen in this study to investigate the effect of some laboratory parameters such as cytokines IL1, IL4, IL6, and IL20 measured by Enzyme-linked Immunosorbent Assay (ELISA) on patients visiting health institutions in the Al-Anbar Governorate / Iraq. The results of the laboratory tests of patients who had symptoms of rheumatoid arthritis were compared with the laboratory test results of healthy people for comparison.

Results: The results indicated that the level of the cytokine IL1 increased significantly in the rheumatoid arthritis patient group (23.24 pg/mL) when compared with the group of healthy people (3.48 pg/mL). The results showed that the level of cytokine IL6 significantly increased in the rheumatoid arthritis patient group (50.66 pg/mL) compared to the healthy group (3.36 pg/mL). The results of cytokine IL20 significantly increased in the rheumatoid arthritis patient group (46.03 pg/mL) compared to the healthy group (15.02 pg/mL). The cytokine IL4 level showed a significant increase within the rheumatoid arthritis patient group (51.95 pg/mL) compared with the healthy people group (9.09 pg/mL).

Conclusion: The levels of cytokines IL1, IL4, IL6, and IL20 increased significantly in the rheumatoid arthritis patient group compared to the group of healthy people.

Keywords: Anti-Inflammatory Agents; Arthritis; Cytokines; Interleukin; Rheumatoid.

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Introduction

Rheumatoid arthritis (RA) is a kind of autoimmune disease that affects the whole body and is characterized by non-articular involvement and inflammatory arthritis. In many instances, it is a chronic inflammatory disease that develops by an interaction between genes and environmental factors. The synovial joints are the primary target of this condition. The condition normally begins in the smaller joints of the periphery. It is typically symmetric, and if it is not treated, it eventually spreads to the joints of the proximal extremities (1). Over time, joint degradation, including bone erosion and cartilage loss, may be caused by an infection. For RA to be diagnosed in the early stages, the symptoms should have persisted for less than six months; and for RA to be considered as established, the symptoms should have persisted for more than six months. Chronic inflammatory disorder is a disease that worsens with time and increases the risk of death if not treated (2). The diagnosis of this condition might be difficult in the early stages of RA, as no laboratory test can identify the presence of the disease. A complete

clinical approach is essential for diagnosing and preventing joint injury that might be disabling. To be effectively treated, patients suffering from RA need treatment that includes both pharmacological and non-pharmacological therapy. The early treatment of rheumatic illness with disease-modifying, anti-rheumatic medications is now considered the standard of therapy. Many patients, despite receiving this therapy, eventually become disabled and have severe morbidity during their illness (3).

This study aimed to explore the impact of the imbalance between pro- and anti-inflammatory cytokines among patients with RA in the Al-Anbar Governorate, Iraq, in comparison with normal healthy controls.

Patients and Methods

This is a case-control study on 150 individuals, two groups were chosen to study the effect of some laboratory parameters, such as cytokines that cause rheumatoid inflammation and cytokines that are anti-rheumatic, for patients visiting health institutions in Al-Anbar Governorate /Iraq for the period from 2nd of Jan. 2023 to the 30th of Aug. 2023, who had symptoms of rheumatoid arthritis, while comparing

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the results of their laboratory tests with the results of healthy people. The first group (control group) consisted of 50 healthy people without rheumatoid arthritis. The second group (patient group) consisted of 100 patients with rheumatoid arthritis. The 100 Iraqi RA cases were diagnosed by a rheumatologist according to the American College of Rheumatology (ACR)1987 criteria or ACR-EULAR (European Alliance of Associations for Rheumatology)2010 criteria. (4) The ages of the patients ranged from 28 to 70 years for both study groups and for both sexes. Five milliliters of blood were drawn from each participant using sterile procedures. Blood samples were centrifuged to separate the serum from the supernatant, and then the supernatant was carefully discarded. The separated serum was moved to Eppendorf tubes, then aliquoted and stored at -20°C until the IL Enzyme-linked Immunosorbent Assay (ELISA) test was performed to measure the serum IL concentration as per the manufacturer's instructions (Shanghai/ China). Information was taken from the patient, which included the patient's age, gender, weight, height, and medical history.

Statistics Analysis: The Mean \pm SD was used for normally distributed numerical variables and the median (interquartile range) was used for non-normally distributed numerical variables in the descriptive statistical analysis. Rates and proportions were calculated for categorical data. The association between socio-demographic characteristics and IL was examined by the Chi-square test and the Fisher exact test. Data of patients were analyzed using SPSS version 25.0 software. Significant results were accepted at the 0.05 level

Results

Demographic characteristics of participants: The two research groups in this study had ages ranging from 28 to 70 years. Details are shown in Table 1. The results of Table 1 indicated that the average age of patients with rheumatoid arthritis was 58.33 years, their ages ranged from 32 to 70 years, while the average age of healthy people was 47.60 years, and their ages ranged from 28 to 65 years.

Table 1: Demographic characteristics of the included participant

| Variable | Group | Mean | No. | SD. | P values |
|--------------------------|----------------------|-------|-----|-------|----------|
| Age (year) | Control | 47.60 | 50 | 9.71 | 0.001 |
| | Rheumatoid arthritis | 58.33 | 100 | 9.48 | |
| BMI (Kg/m ²) | Control | 27.25 | 50 | 3.28 | 0.001 |
| | Rheumatoid arthritis | 38.60 | 100 | 3.79 | |
| ESR (mm/hr) | Control | 15.34 | 50 | 2.57 | 0.001 |
| | Rheumatoid arthritis | 24.24 | 100 | 7.19 | |
| IL-1 (pg/mL) | Control | 3.48 | 50 | 0.95 | 0.001 |
| | Rheumatoid arthritis | 23.24 | 100 | 6.68 | |
| IL-6 (pg/mL) | Control | 3.36 | 50 | 0.92 | 0.001 |
| | Rheumatoid arthritis | 50.66 | 100 | 12.04 | |
| IL-20 (pg/mL) | Control | 15.02 | 50 | 2.38 | 0.001 |
| | Rheumatoid arthritis | 46.03 | 100 | 10.65 | |
| IL-4 (pg/mL) | Control | 9.09 | 50 | 4.56 | 0.001 |
| | Rheumatoid arthritis | 51.95 | 100 | 11.99 | |

Table 1 showed that women are more susceptible to rheumatoid arthritis, as 66 women out of a total of 100 cases were recorded, while the number of infected males was 34 males.

Table 1 indicated that the two study groups differed in average BMI values, as the patient group had higher BMI values, recording 38.60 kg/m² compared to 27.25 kg/m² for the control group.

The values of the patient group indicated that patients with rheumatoid arthritis suffer from obesity, while the values of the control group were within normal ranges.

Laboratory tests: Table 1 shows that the two tested groups differed significantly in the ESR values. The group of patients with rheumatoid arthritis achieved a significant increase in the ESR value (P value \leq 0.001), reaching 24.24 mm/hr, while the control group recorded 15.34 mm/hr. The elevation of ESR value in patients with rheumatoid arthritis may indicate the presence of an inflammatory process in the body.

Table 1 indicated that the level of the cytokine IL1 increased significantly in the group of patients with rheumatoid arthritis to 23.24 pg/mL. While the control group (healthy people) recorded 3.48 pg/mL (P value \leq 0.001).

Table 1 showed that the average level of the cytokine IL-6 for the two groups studied differed significantly in its values. The group of patients with rheumatoid arthritis achieved a significant increase of 50.66 pg/mL, while the group of healthy persons recorded 3.36 pg/mL, (P value \leq 0.001).

The results of measuring the levels of the cytokine IL20 in the two study groups showed that the level of the cytokine had increased significantly in the group of rheumatoid arthritis patients compared to the group of healthy people (46.03 vs. 15.02) pg/mL, (P value \leq 0.001).

Table 1 showed that the level of the cytokine IL4 showed significant superiority within the rheumatoid arthritis patient population, where it was recorded at 51.95 pg/mL, while the cytokine level was 9.09 pg/mL in the group of people without rheumatoid arthritis, (P value \leq 0.001).

Discussion

Demographic characteristics of participants: The results of this table were consistent with the results of the study conducted by researcher Kato et al. (2017) which showed that the average age of patients with rheumatoid arthritis was 55.8 years during the year 2002-2003, 57.0 years during the year 2007-2008, and 59.9 years during the year 2012-2013(5).

In a study conducted in 2023 by Yu and others, it was shown that the risk of developing rheumatoid arthritis is greatly affected by patient's age (6). The study showed that early detection of the disease and treatment in the early stages impact controlling the disease and reducing its complications. It also showed that health education and health programs that aim to control the disease in middle-aged patients have a major role in treating the disease. According to Radu *et al.* (2021) risk factors for developing rheumatoid arthritis are age, gender, heredity, and environmental stressors such as smoking and air pollutants(7).

Also, Maranini *et al.* (2022) indicated that Rheumatoid arthritis is a long-term inflammatory condition that impacts females with a ratio of 3:1 (female/male) (8). The role of gender in the susceptibility of females to rheumatoid arthritis may be due to hormonal factors and the effect of sexual dysmorphism (9). Alpizar-Rodriguez (2019) indicated that pregnancy and breastfeeding have been linked with a decreased risk of rheumatoid arthritis (10).

In a study conducted by Abuhelwa *et al.* (2020) showed that out of a total of 5428 people, 32.8% were above normal weight (overweight), 30.4% were obese, and 33.9% had a normal BMI (11). Feng X *et al.* (2019) showed that the elevation in BMI was associated with an increasing risk of rheumatoid arthritis development (12).

Laboratory findings

In a study by Jassim *et al.* (2021) it was concluded that there is a significant increase in ESR values for the rheumatoid arthritis patient group in comparison to healthy people (13).

IL1 contributes to many inflammatory diseases by initiating and enhancing immune and inflammatory responses (14). It plays a role in several systemic autoinflammatory syndromes and juvenile rheumatoid arthritis.

It also contributes a pathogenic role in inflammation and tissue destruction. It is a cytokine made up of eleven structurally similar proteins, involved in or controlling inflammation, which acts primarily by binding to specific receptors on the plasma membrane of target cells (15).

It has been observed that the cytokine IL6 mainly in the neurological and cardiovascular systems is accountable for the systemic symptoms of rheumatoid arthritis. In general, patients with rheumatoid arthritis have been seen to have elevated

serum levels of IL6 and IL6R in the serum and synovial fluid of the affected joints (16). IL6 is

produced by infections and other types of inflammation directly, mostly by macrophages in response to infections or molecular patterns associated with inflammation. IL6 removes infectious organisms and promotes the acute phase and immunostaining reactions to mend injured tissue, acting as a protective measure. For both innate and adaptive immunity, IL6 is essential (17).

The role of the cytokine IL20 is to participate in the process of synovial angiogenesis in rheumatoid arthritis because it stimulates the expression of many angiogenic mediators such as fibroblast growth factor 2, vascular endothelial growth factor, and MMP in endothelial cells, thus promoting the infiltration of synovial tissue with Immunological cells (18). IL20 has important inflammatory effects through leukocytes chemotaxis to the synovial membrane and sites of bone erosion in particular. IL20 also stimulates the production of several cytokines involved in the inflammatory process, including IL6, IL8, matrix metalloproteinase (MMP), and monocyte chemoattractant protein (MCP)-1, leading to joint inflammation and destruction (19). Hussein *et al.* (2022) found that the level of IL20 significantly increased in patients with RA which recorded 58.5 ng/L as compared with 15.1 ng/L for healthy controls and they concluded that the cytokines IL20 was correlated with disease activity of rheumatoid arthritis (20).

The results of Table 1 were consistent with the results reached by Giri *et al.* (2021) in which they confirmed that IL4 levels were high in patients with rheumatoid arthritis compared to people who do not have the disease (21). Rheumatoid arthritis is now suspected to be driven by pathogenic Th17 cells that secrete interleukin 17 (IL17) and may be regulated by IL4 (22).

Limitation: Insufficient sample size for statistical measurements.

Conclusions:

In this study, the results indicated that the level of the cytokine IL1, cytokine IL6, cytokine IL20, and cytokine IL4, increased significantly in the rheumatoid arthritis patient group, compared to the group of healthy people. There is imbalance between pro- and anti-inflammatory cytokines among patients with RA in comparison with normal healthy controls.

Authors' declaration:

We confirm that the table presented in the manuscript is our original work.

The project was approved by the local ethical committee in the College of Science, University of Çankiri according to the code number (47-03.12.11.2021).

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Authors' contributions

Study conception & design: (Shakir M. Salih, Sevki Adam). Literature search: (Shakir M. Salih). Data acquisition: (Shakir M. Salih). Data analysis & interpretation: (Shakir M. Salih). Manuscript preparation: (Shakir M. Salih). Manuscript editing & review: (Shakir M. Salih, Sevki Adam).

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اختلال السيتوكينات المؤيدة والمضادة للالتهابات في التهاب المفاصل الروماتويدي لدى المرضى العراقيين

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الخلاصة:

خلفية البحث: التهاب المفاصل الروماتويدي (RA) هو مرض التهابي مزمن مناعي ذاتي يصيب الجسم كله ويتميز بمشاكل غير مفصلي وغير مفصلي. في كثير من الحالات، يحدث المرض من خلال التفاعل بين الجينات والعوامل البيئية.

الاهداف: تم تصميم هذا البحث للتحقيق في تأثير عدم التوازن بين السيتوكينات المؤيدة والمضادة للالتهابات في المرضى الذين يعانون من التهاب المفاصل الروماتويدي ومقارنة نتائجهم مع الأشخاص الأصحاء الذين لا يعانون من المرض.

طرق العمل: تم اختيار مجموعتين (مائة) مريض يعانون من التهاب المفاصل الروماتويدي (66 أنثى، 34 ذكر) و 50 مجموعة مراقبة صحية (28 أنثى، 22 ذكر) في هذه الدراسة للتحقيق في تأثير بعض المعلمات المختبرية - مثل السيتوكينات IL1 و IL4 و IL6 و IL20 التي تم قياسها بواسطة مقاييسه المتميز المناعي المرتبط بالإنزيم (ELISA) - على المرضى الذين يزورون المؤسسات الصحية في محافظة الأنبار / العراق. تمت مقارنة نتائج الاختبارات المعملية للمرضى الذين يعانون من أعراض التهاب المفاصل الروماتويدي مع نتائج الاختبارات المعملية للأشخاص الأصحاء - للمقارنة.

النتائج: أشارت النتائج إلى أن مستوى السيتوكين IL1 زاد معنويًا في مجموعة مرضى التهاب المفاصل الروماتويدي (23.24 pg/mL) بالمقارنة مع مجموعة الأشخاص الأصحاء (3.48 pg/mL). أظهرت النتائج أن مستوى السيتوكين IL6 زاد معنويًا في مجموعة مرضى التهاب المفاصل الروماتويدي (50.66 pg/mL) مقارنة بالمجموعة السليمة (3.36 pg/mL). زادت نتائج السيتوكين IL20 بشكل معنوي في مجموعة مرضى التهاب المفاصل الروماتويدي (46.03 pg/mL) مقارنة بالمجموعة السليمة (15.02 pg/mL). أظهر مستوى السيتوكين IL4 زيادة معنوية داخل مجموعة مرضى التهاب المفاصل الروماتويدي (51.95 pg/mL) مقارنة بمجموعة الأشخاص الأصحاء (9.09 pg/mL).

الاستنتاجات: ارتفع مستوى السيتوكين IL1 و IL4 و IL6 و IL20 بشكل ملحوظ في مجموعة مرضى التهاب المفاصل الروماتويدي مقارنة بمجموعة الأشخاص الأصحاء.

الكلمات المفتاحية: العوامل المضادة للالتهابات، التهاب المفاصل، السيتوكينات، الإنترلوكين، الروماتويد.