Ibn Al-Haitham Journal for Pure and Applied Sciences
Journal homepage: jih.uobaghdad.edu.iq
PISSN: 1609-4042, EISSN: 2521-3407
IHJPAS. 2024, 37(4)

Immunological Role of IL-3, IL-5 and Some Inflammation Markers in a Sample of Iraqi Patients with Rheumatoid Arthritis

Shams A. Salman[1,*] and Rakad M. Kh AL-Jumaily[2]
[1,2]Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq
*Corresponding Author.

Received: 14 May 2023 Accepted: 19 June 2023 Published: 20 October 2024
doi.org/10.30526/37.4.3493

Abstract

Rheumatoid arthritis (RA) occurs through joint inflammation and affects approximately one in 200 adults worldwide, with women affected two to three times more frequently than men. The design of this study is to examine serum levels and changes in immunological markers in Iraqi patients with RA. To evaluate the advantages of clinical biomarkers of autoimmune disorderliness as well as changes in some immunological markers and clinical outcomes associated with Iraqi rheumatoid arthritis patients. Interleukins-3 and 5 (IL-3 and IL-5) were quantified by ELISA assay kits in the serum of 60 patients with RA disease (age range 20–60 years) and 30 age-matched healthy control groups. The BMI (body mass index), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), anti-cyclic citrullinated peptide (anti-CCP), and complete blood count (CBC) were also measured. The results of IL-3 and IL-5 (341.56 ±12.10 and 504.28 ±25.20) levels displayed a significant increase in RA patients compared to control (154.66 ±9.24 and 117.61 ±3.73). The levels of ESR and Ant-CCP (37.60 ±2.72 and 0.637 ±0.02) increased significantly in RA patients as compared to control (8.12 ±1.14 and 0.308 ±0.01). Similar results were observed (11.98 ±1.18) with CRP levels in RA patients. The results of CBC indicated a significant reduction in hemoglobin (HB) levels (12.42 ±0.34) in RA patients, and there were no differences between other CBC parameters compared to healthy controls. These findings suggest that it is possible to use IL-3 and IL-5 as biomarkers for RA diagnosis.

Keywords: Rheumatoid arthritis, interleukins-3, interleukins-5, C-reactive protein.

1. Introduction

Rheumatoid arthritis (RA) is an inflammatory disease marked by dysregulation of the immune system brought on by a complex interaction between environmental and genetic factors [1]. Aberrant adaptive immunity is currently thought to be the driving force behind the progression of rheumatoid arthritis (RA) from preclinical illness to obvious synovitis. In severe cases, joint inflammation can cause permanent joint damage and disability. The lungs, heart, blood vessels, skin, and eyes are just a few of the organs that could be affected. Rheumatoid arthritis affects approximately one in 200 adults worldwide, and women are affected two to three times more frequently than men [2, 3]. During the pathogenesis of RA, a number of immune cells migrate

into the joint tissues activating resident cells like synovial fibroblasts, chondrocytes, osteoblasts, and osteoclasts. These immune cells include macrophages, dendritic cells, lymphocytes, and neutrophils. There are three overlapping mechanisms in this procedure: inflammation, synovial hyperplasia, and an altered immune response [4]. According to [5], rheumatoid arthritis patients commonly present with joint agony and stiffness across various joints. However, the most frequently impacted joints are the metacarpophalangeal, proximal interphalangeal, and wrist [6, 7]. The name interleukin-3 was primarily used to describe a T-cell-derived lymphokine that induces the expression of 20-alpha hydroxysteroid dehydrogenase (SDH) in cultures of nude mouse splenic lymphocytes. Recent research shows that IL-3 has different biological properties and mechanisms by which it promotes differentiation and hematopoietic cell growth [8, 9]. These operations are controlled by a number of cytokines produced by some cells, including activated T cells, macrophages, and stromal cells. Interleukin 3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) are two of these cytokines that stimulate multiple hematopoietic cell lineages, and depending on the target cells, IL-3 performs a variety of biological functions because they target a broad range of cells. On the other hand, IL-5 mostly encourages eosinophil colony formation and some murine B cell proliferation and differentiation [10, 11]. However, the information about the role of IL-3 and IL-5 in the pathogenesis of RA is still very poor, but it suggests that IL-3 has a potential role in the regulation of the inflammatory response and bone and cartilage loss in the arthritis of mice [12]. The erythrocyte sedimentation rate (ESR) is one of the hematological tests used to determine body-inflammatory activity. The presence of inflammation is indicated by an increase in the rate of settling of red blood cells (RBCs) in the blood sample's test tube, which is measured by this analysis. It is important to note that, despite the fact that ESR is a nonspecific analysis, the location of inflammation was not found [13]. C-reactive protein can be utilized as a timely marker of active inflammation, making it preferable to the ESR in inflammatory conditions [14, 15]. Numerous studies have demonstrated a correlation between C-reactive protein levels and an increased rate of radiological progression in rheumatoid arthritis [16, 17]. Anti-CCP is one of the anti-flagging family members that interacts with the antigenic determinant containing citrullinated arginine residues. Ant-CCP has been used for many years along with rheumatoid factor (RF) to help diagnose RA [18]. This study aimed to examine the changes in immunological markers at serum levels as well as the clinical outcomes associated with these markers in Iraqi rheumatoid arthritis patients.

2. Materials and methods

2.1 Study groups

This study was performed on sixty RA patients with an age range of 20–70 years and 30 healthy controls with an age range of 20–60 years. Patients were admitted to the Al-Yarmouk Teaching Hospital, Baghdad, Iraq, from October to December 2022. The diagnosis was made by the consulting medical staff.

2.2 Blood collection

Venous (5 ml each) blood samples were collected into a serum gel- tube from RA patients and controls. The tube was centrifuged at 5000 rpm for ten minutes after clot formation at room temperature. The serum was kept in 1.5 ml Eppendorf tubes at a freezing temperature of -20℃ until use for the serological test.

2.3 Measurements of studied markers

Interleukin-3 and interleukin-5 were assessed quantitatively in serum samples using commercial enzyme-linked immunosorbent assay (ELISA) kits (Sun Long, China). According to the manufacturer's guidelines. The analysis of ESR and CBC was done by adding 2 ml of blood sample inside an ethylene diamine tetra acetic acid (EDTA) tube, which is a vial specially designed to prevent clotting blood. The sample was then put into a machine for analysis. One drop of serum for the CRP test is put on a circle on a glass slide. One drop of reagent latex was added to all the circles with separate sticks, mixing and spreading the fluid over the area of the cell back and forth for 2 minutes, slowly agglutinating, and observing under artificial light, preferably.

2.4 Statistical analysis

The statistical analysis program (SPPS) version 23 was used to test the differences between the means of the study groups. A statistical comparison between groups was analyzed using a T-test and P <0.05 was considered a significant value.

3. Results

Interleukin-3 and IL-5 were significantly increased in RA patients as compared to the control group (**Table 1**).

Table 1. Comparison between RA patients and control groups in IL-3 and IL-5.

Groups	IL-3 (Pq/ml)	IL-5 (Pq/ml)
Patients	341.56 ±12.10	504.28 ±25.20
Control	154.66 ±9.24	117.61 ±3.73
T-test	33.88 **	68.697 **
P-value	0.0001	0.0001

Data are presented as mean ± standard error (SE). **P<0.01.

The WBC levels didn't differ significantly in RA patients compared to healthy controls, as illustrated in **Table 2**. Also, lymphocyte levels didn't differ significantly in RA patients compared to healthy controls, as indicated in **Table 2**. Monocyte levels were significantly higher in patients with RA as compared to the control group. In contrast, the RBC levels were significantly lower in RA patients as compared to the control group. The results of **Table 2** also indicated that HGB levels were significantly different in RA patients than in the control group.

Table 2. A comparative analysis of WBC, Lymph, Mono, RBC and HGB between studies groups.

Groups	WBC	Lymphocyte%	Monocyte%	RBC	HGB
Patients	7.64 ±0.41	33.04 ±1.60	8.84 ±0.35	4.81 ±0.09	12.42 ±0.34
Control	6.24 ±0.39	26.83 ±2.72	6.82 ±0.30	5.36 ±0.14	13.81 ±0.34
T-test	1.470 NS	6.3716 NS	1.278 **	0.376**	1.246 *
P-value	0.0632	0.0571	0.0025	0.0052	0.0296

Data are presented as mean ± standard error (SE). * P≤0.05; **P≤0.01. NS: Non-Significant

The statistical testing showed there was no significant change in the IL-3 levels among the male and female RA patients **(Table 3)**. Conversely, a significant difference in the level of IL-5 among the male and female RA patients was observed, as illustrated in **Table 3**.

Table 3. Effect of Gender in parameters of sample study.

Gender	IL-3 (Pq/ml)	IL-5 (Pq/ml)
Male	331.96 ±24.69	574.04 ±50.49
Female	346.58 ±13.52	473.18 ±28.56
P-value	0.574 NS	0.050 *

Data are presented as mean ± standard error (SE). * P≤0.05; NS: Non-Significant

Group of RA patients was split into three groups based on age (20–40 years, 41–60 years and >60 years), as shown in **Table 4**. The statistical analysis indicated there was no significant difference in the IL-3 levels among different age groups of RA patients. However, the level of IL-5 indicated significant differences between the (20–40 yr.) and (40–60 yr., >60 yr.) groups **(Table 4)**.

Table 4. Effect of Age groups in parameters of sample studied.

Groups	IL-3 (Pq/ml)	IL-5 (Pq/ml)
20-40 yr.	331.96 ± 24.68	613.38 ± 49.64 a
41-60 yr.	342.35 ± 14.62	467.41 ± 28.85 b
>60 yr.	386.77 ± 6.41	416.36 ± 102.72 b
P-value	0.595 NS	0.032 *

Data are presented as mean ± standard error (SE). Different letters in same column referred to significant value. * (P≤0.05).

The levels of ESR and Ant-CCP increased significantly in RA patients as compared to controls. Similar results were also observed with CRP levels in RA patients, as illustrated in **Table 5**.

Table 5. A comparative analysis of ESR, CRP and Anti-CCP between studies groups.

Groups	ESR(mm/h)	CRP(mg/l)	Anti-CP(U/ML)
Patients	37.60 ±2.72	11.98 ±1.18	0.637 ±0.02
Control	8.12 ±1.14	4.83 ±0.38	0.308 ±0.01
T-test	7.490 **	3.511**	0.0735 **
P-value	0.0001	0.0001	0.0001

Data are presented as mean ± standard error (SE). **P≤0.01.

4. Discussion

The results of this study indicated that IL-3 and IL-5 were significantly higher in RA patients as compared to the control group. Interleukin-3 is a cytokine secreted by specific immune cells called Th cells. Interleukin-3 has a role in links between the immune response and the hematopoietic system. It has been suggested that IL-3 has a potent inhibitory role in inflammatory arthritis and pathological bone resorption [19, 20]. In addition, IL-3 plays a vital role in modulating Treg cell development under different conditions. Similar results were obtained by investigating the role of IL-5 in stimulating rheumatoid arthritis in specific cases [21-23]. To better understand the role of IL-3 and IL-5 in the regulation of immune responses in RA, more investigation is necessary. Also, the results showed the lymphocyte levels didn't differ significantly in RA patients compared to the controls. However, these findings disagree with [24] results that showed the neutrophil-to-lymphocyte ratio (NLR) was significantly increased in RA patients, and no significant difference was found between male and female RA patients with

regard to the NLR ratio. Measures of anemia and proinflammatory cytokines in RA patients are correlated. The overall levels of hemoglobin were inversely correlated with the levels of some interleukins, such as IL-6. This study shows HGB levels were significantly lower in RA patients than in healthy controls. The RA disease is common and frequently involves anemia. However, a recent review suggested that regardless of the underlying illness process that causes the anemia, untreated anemia is possible to have severe clinical results practically in RA disease [25-27]. This study indicated that levels of ESR, CRP, and anti-CCP were significantly higher in RA patients than in the control group. Many studies have suggested that CRP and ESR are considered clear markers for RA. However, concerning the relationship between RA and CRP concentrations, contradictory findings have been presented. The disparity might be made sense of by the likelihood that CRP is a great-stage protein in the liver that is extremely delicate to momentary changes in aggravation. CRP concentrations suggest they may not rise in RA patients through chronic, long-term inflammation. The level of ESR, on the other hand, is an indicator of acute-phase protein and is indirect and slowly responds to stimulation. It is also sensitive to non-acute-phase proteins such as rheumatoid factor and may be an indicator better for the assessment of subclinical inflammation than CRP [28, 29]. In addition, it suggests that a healthy individual with a positive anti-CCP antibody test runs a significant risk of developing RA in the future [30,31].

5. Conclusions

These findings suggest that it is possible to use IL-3 and IL-5 as biomarkers for RA diagnosis.

Conflict of Interest

There is no conflict of interest.

Funding

There is no funding for the article.

Ethical Clearance

This study was authorized by the Department of Biology, College of Science, University of Baghdad's ethical committee (Ref.: CSEC/0922/0111, September 29, 2022). This study was guided by the Declaration of Helsinki, the code of ethical principles for medical studies using human subjects.

References

1. Wang, X.; Fan, D.; Cao, X.; Ye, Q.; Wang, Q.; Zhang, M.; Xiao, C. The role of reactive oxygen species in the rheumatoid arthritis-associated synovial microenvironment. *Antioxidants* **2022**, *11*, 1153. https://doi.org/10.3390/antiox11061153.
2. Farhat, H.; Irfan, H.; Muthiah, K.; Pallipamu, N.; Taheri, S.; Thiagaraj, S.S.; Shukla, T.S.; Gutlapalli, S.D.; Giva, S.; Penumetcha, S.S. Increased Risk of Cardiovascular Diseases in Rheumatoid Arthritis: A Systematic Review. *Cureus* **2022**, *14(12)*, e32308. https://doi.org/10.7759/cureus.32308.
3. Smith, M.H. and Berman, J.R. What is rheumatoid arthritis? *JAMA* **2022**, *327*, 1194-1194 https://doi.org/10.1001/jama.2022.0786.
4. Fang, Q.; Zhou, C.; Nandakumar, K.S. Molecular and cellular pathways contributing to joint damage in rheumatoid arthritis. *Mediators of Inflammation* **2020**, 3830212. https://doi.org/10.1155/2020/3830212.

5. Littlejohn, E.A.; Monrad, S.U. Early diagnosis and treatment of rheumatoid arthritis. *Primary Care: Clinics in Office Practice* **2018**, *45*, 237-255. https://doi.org/10.1016/j.pop.2018.02.010.
6. Carpanen, D.; Kedgley, A.E.; Shah, D.S.; Edwards, D.S.; Plant, D.J.; Masouros, S.D. Injury risk of interphalangeal and metacarpophalangeal joints under impact loading. *Journal of the Mechanical Behavior of Biomedical Materials* **2019**, *97*, 306-311. https://doi.org/10.1016/j.jmbbm.2019.05.037.
7. Mo, Y.-Q.; Yang, Z.-H.; Wang, J.-W.; Li, Q.-H.; Du, X.-Y.; Huizinga, T.; Matthijssen, X.; Shi, G.-Z.; Shen, J.; Dai, L. The value of MRI examination on bilateral hands including proximal interphalangeal joints for disease assessment in patients with early rheumatoid arthritis: a cross-sectional cohort study. *Arthritis Research & Therapy* **2019**, *21*, 1-12. https://arthritis-research.biomedcentral.com/articles/10.1186/s13075-019-2061-1.
8. Srivastava, R.K.; Tomar, G.B.; Barhanpurkar, A.P. IL-3 attenuates collagen-induced arthritis by modulating the development of Foxp3+ regulatory T cells. *Journal of Immunology* **2011**, *186(4)*, 2262-2272. https://doi.org/10.4049/jimmunol.1002691.
9. Ihle, J.N. Interleukin-3 and hematopoiesis. *Chemical immunology* **1992**, *51*, 65-106. https://doi.org/10.1159/000420755.
10. Kawabata, H.; Satoh, M.; Yatera, K. Development of Rheumatoid Arthritis During Anti-Interleukin-5 Therapy in a Patient with Refractory Chronic Eosinophilic Pneumonia. *Journal of Asthma Allergy* **2021**, *14*, 1425-1430. https://doi.org/10.2147/jaa.s342993.
11. Hara, T.; Miyajima, A. Function and signal transduction mediated by the interleukin receptor system in hematopoiesis. *Stem Cells* **1996**, *14*, 605-618. https://doi.org/10.1002/stem.140605.
12. Pelaia, C.; Paoletti, G.; Puggioni, F.; Racca, F.; Pelaia, G.; Canonica, G.W.; Heffler, E. Interleukin-5 in the pathophysiology of severe asthma. *Frontiers in Physiology* **2019**, *10*, 1514. https://doi.org/10.3389/fphys.2019.01514.
13. Diwan, Z. J.; Al-Marsomy, W.A. Evaluation of Several Hematologic and Serological Parameters in Rheumatoid Arthritis Patients. *Ibn AL-Haitham Journal For Pure and Applied Sciences* **2024**, *37(2)*, 129-135. https://doi.org/10.30526/37.2.3451.
14. Orr, C.K.; Najm, A.; Young, F.; McGarry, T.; Biniecka, M.; Fearon, U.; Veale, D.J. The Utility and Limitations of CRP, ESR and DAS28-CRP in Appraising Disease Activity in Rheumatoid Arthritis. *Front Medical (Lausanne)* **2018**, *5*, 185. https://doi.org/10.3389/fmed.2018.00185.
15. Breda, L.; Nozzi, M.; De Sanctis, S.; Chiarelli, F. Laboratory tests in the diagnosis and follow-up of pediatric rheumatic diseases: an update. *In Proceedings of the Seminars in Arthritis and Rheumatism*, **2010**, 53-72. https://doi.org/10.1016/j.semarthrit.2008.12.001.
16. Jonsson, M.K.; Sundlisæter, N.P.; Nordal, H.H.; Hammer, H.B.; Aga, A.-B.; Olsen, I.C.; Brokstad, K.A.; van der Heijde, D.; Kvien, T.K.; Fevang, B.-T.S. Calprotectin as a marker of inflammation in patients with early rheumatoid arthritis. *Annals of the Rheumatic Diseases* **2017**, *76*, 2031-2037. https://doi.org/10.1136/annrheumdis-2017-211695.
17. Shmerling, R.H. Testing for Anti–Cyclic Citrullinated Peptide Antibodies: Is It Time to Set This Genie Free? *Archives of Internal Medicine* **2009**, *169*, 9-14. https://doi.org/10.1001/archinternmed.2008.522.
18. Avouac, J.; Gossec, L.; Dougados, M. Diagnostic and predictive value of anti-cyclic citrullinated protein antibodies in rheumatoid arthritis: a systematic literature review. *Annals of the Rheumatic Diseases* **2006**, *65*, 845-851. https://doi.org/10.1136/ard.2006.051391.
19. Omran, R.H.; Ahmed, Z.A.; Alrawi, A.A. Evaluation of Some New Cytokines in Rheumatoid Arthritis. *Journal of Faculty of Medicine Baghdad* **2022**, *64*, 3. https://doi.org/10.32007/jfacmedbagdad.6431945.
20. Srivastava, R.K.; Tomar, G.B.; Barhanpurkar, A.P.; Gupta, N.; Pote, S.T.; Mishra, G.C.; Wani, M.R. IL-3 attenuates collagen-induced arthritis by modulating the development of Foxp3+ regulatory T cells. *The Journal of Immunology* **2011**, *186*, 2262-2272. https://doi.org/10.4049/jimmunol.1002691.

21. Kawabata, H.; Satoh, M. ; Yatera, K. Development of Rheumatoid Arthritis During Anti-Interleukin-5 Therapy in a Patient with Refractory Chronic Eosinophilic Pneumonia. *Journal of Asthma and Allergy* **2021**, 1425-1430. https://doi.org/10.2147/JAA.S34299.
22. Mikhaylenko, D.S.; Nemtsova, M.V.; Bure, I.V.; Kuznetsova, E.B.; Alekseeva, E.A.; Tarasov, V.V.; Lukashev, A.N.; Beloukhova, M.I.; Deviatkin, A.A.; Zamyatnin, A.A. Jr. Genetic Polymorphisms Associated with Rheumatoid Arthritis Development and Antirheumatic Therapy Response. *International Journal of Molecular Science* **2020**, *21(14)*, 4911. https://doi.org/10.3390/ijms21144911.
23. Brühl, H.; Cihak, J.; Niedermeier, M.; Denzel, A.; Gomez, M.R.; Talke, Y.; Goebel, N.; Plachý, J.; Stangassinger, M.; Mack, M. Important role of interleukin-3 in the early phase of collagen-induced arthritis. *Arthritis & Rheumatism* **2009**, *60*, 1352-1361. https://doi.org/10.1002/art.24441.
24. Fawzy, R.M.; Said, E.A.; Mansour, A.I. Association of neutrophil to lymphocyte ratio with disease activity indices and musculoskeletal ultrasound findings in recent onset rheumatoid arthritis patients. *The Egyptian Rheumatologist* **2017**, *39*, 203-206. http://dx.doi.org/10.1016/j.ejr.2017.05.001.
25. Wilson, A.; Yu, H.-T.; Goodnough, L.T.; Nissenson, A.R. Prevalence and outcomes of anemia in rheumatoid arthritis: a systematic review of the literature. *The American Journal of Medicine* **2004**, *116*, 50-57. https://doi.org/10.1016/j.amjmed.2003.12.012.
26. Yoshida Y, Tanaka T. Interleukin 6 and rheumatoid arthritis. *Biomedical Research International* **2014**, *2014*, 698313. DOI: https://doi.org/10.1155/2014/698313.
27. Avci, A.B.; Feist, E.; Burmester, G.R. Targeting IL-6 or IL-6 Receptor in Rheumatoid Arthritis: What Have We Learned?. *BioDrugs*, **2024**, *38*, 61–71. https://doi.org/10.1007/s40259-023-00634-1.
28. Wu, Y.; Chen, Y.; Yang, X.; Chen, L.; Yang, Y. Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were associated with disease activity in patients with systemic lupus erythematosus. *International Immunopharmacology* **2016**, *36*, 94-99. https://doi.org/10.1016/j.intimp.2016.04.006.
29. Pope, J.E.; Choy, E.H. C-reactive protein and implications in rheumatoid arthritis and associated comorbidities. *Seminars in Arthritis and Rheumatism* **2021**, *51(1)*, 219-229. https://doi.org/10.1016/j.semarthrit.2020.11.005.
30. Peng, Y.-F.; Cao, L.; Zeng, Y.-H.; Zhang, Z.-X.; Chen, D.; Zhang, Q.; Zhu, Y.-S. Platelet to lymphocyte ratio and neutrophil to lymphocyte ratio in patients with rheumatoid arthritis. *Open Medicine* **2015**, *10*. https://doi.org/10.1515/med-2015-0037.
31. Nazar, L.A.; Al-Aati, E. A. Study of IL-33 and IL-1R4 in Iraqi Rheumatoid Arthritis Female Patient's with and without Dyslipidemia Prone to Atherosclerosis. *Ibn AL-Haitham Journal For Pure and Applied Sciences* **2019**, *32(1)*, 48-56. https://doi.org/10.30526/32.1.1920.