AL-KUFA JOURNAL FOR BIOLOGY
**ISSN (Print):** 2073-8854 **ISSN (online):** 2311-6544

Original Research Paper

# Correlation Between Serum IL-1β Level in Rheumatoid Arthritis Patients and Some Clinical and Sociodemographic Characteristics

**Hussein A. Salih[1], Karrar S. Zayed[1]**

[1]*Department of Pathological Analysis, Faculty of Science, University of Kufa, Kufa, Iraq.*

*Article history*
Received: 7/7/2024
Revised: 20/8/2024
Accepted: 1/9/2024

*Corresponding Author: *Hussein A. Salih, Department of Pathological Analysis, Faculty of Science*, University of Kufa, Kufa, Iraq;
Email: husseina.alfahm@student.uokufa.edu.iq
**DOI: 10.36320/ajb/v16.i3.17234**

**Abstract:** IL-1β is a potent pro-inflammatory cytokine, mainly produced by activated macrophages and monocytes. It induces adhesion molecules, promotes increased leukocyte infiltration, and releases other pro-inflammatory mediators. High levels of IL-1β have been found in RA patients, both in synovial fluid and serum. This study aimed to examine the serum level of IL-1β in RA patients compared with controls and their association with clinical and sociodemographic characteristics. Seventy-one patients with RA and forty-six healthy controls were recruited from the Iraqi Arab population in this case-control study. Measurement of the serum IL-1β level was done in this study using an Enzyme-Linked Immunosorbent Assay kit. According to the analysis, these results indicated that there is a significant increase in the serum level of IL-1β in RA patients compared to that in the control, ($p = 0.039$). Also, IL-1β concentration in RA smoker patients increased significantly compared to RA non-smoker patients ($p=0.045$). In conclusion, serum IL-1β levels were significantly associated with RA disease in the Iraqi Arab population and may regarded as a diagnostic marker for RA diagnosis especially in smokers' patients.

**Keywords:** IL-1β, Rheumatoid arthritis, ELISA, Sociodemographic

## 1. Introduction

Rheumatoid arthritis (RA) is an extremely common, complex systemic, and multivariate autoimmune disease [1]. Inflammation of joints is a constant sign in RA. This leads to fracturing and degeneration of bones [2]. Nearly 0.5-1% of the world's population suffers from this particular RA. In that too, the cases reported in women are more than in men [3]. Genetic predisposition, environmental triggers, and dysregulated immune responses constitute the causes of RA. Its pathogenesis centrally lies in an overproduction of proinflammatory cytokines, which not only further fuels the inflammatory milieu within joints but also systemically contributes to manifestations of this disease. Of these cytokines, interleukin-1 beta (IL-1β) plays a very vital role in the pathogenesis of the inflammation processes that take place in RA. IL-1β is considered one of the main factors for inflammation and is made mainly by stimulated monocytes and macrophages, but also from synovial cells, B cells, and T cells. IL-1β activates monocytes and macrophages and increases inflammation by inducing the production of proinflammatory cytokines like TNF-α and IL-6, which intensify the inflammatory response in the synovium. It promotes the proliferation of fibroblasts in the synovial lining membrane [5]. IL-1β damages the cartilage through the activation of chondrocytes, and it also activates osteoclasts that promote bone resorption. The increased levels of IL-1β in the synovial fluid and plasma of RA patients are associated with multiple indicators of disease activity, also severity [6]. IL-1β in RA enhances the synthesis of prostaglandins, nitric oxide, and chemokines; sensitizes nociceptors; and

**DOI: 10.36320/ajb/v16.i3.17234**

activates endothelial cells, which would lead to an increased inflammation, augmented vascular permeability, and heightened sensitivity in joints, hence aggravating pain and swelling [7].

Multiple studies revealed the correlation of IL-1β with people who suffer from RA [8,9] due to its importance in the therapy. Many researchers, in particular, targeting cytokines or their receptors, for example, TNF-α and IL-6, have set a benchmark in treating patients with RA that do not respond to therapy with DMARDs [9,10].

The current study intended to identify the association of IL-1β with RA disease and some clinical and sociodemographic features among the Iraqi Arab population.

## 2. Methodology

*Samples collection*

This research was conducted on Iraqi Arab patients with RA disease who had been identified in the Rheumatology department in Al-Sader Medical City in Najaf. This study was accomplished with 117 people in total, including 71 patients diagnosed with RA and 46 people are considered as control. They are determined by a specialized rheumatologist using the 2010 guidelines set out by the European League Against Rheumatism and the American College of Rheumatology, as well as serological examinations (ESR, CRP, RF, and ACCP). This study excluded many illnesses like autoimmune diseases (scleroderma, systemic lupus erythematosus, and Sjogren's syndrome), chronic inflammatory diseases (Crohn's disease and ulcerative colitis), infectious diseases (caused by viruses, bacteria, and parasites), cancerous tumors (This might be attributed to the potential of tumors to change cytokine levels and the response of the immune system in consequence), persistent renal and liver disease (They may affect the levels of cytokines in the body), cardiovascular diseases, chronic psychiatric illnesses (which may affect the immune system through chronic stress), and metabolic diseases (thyroid disease)

Amongst the subjects, 52 female patients were included and 19 male patients, varying in age between (21 – 70) years old. Whereas there were about 31 females and 15 males as control people, ranging from (21 – 68) years old. Each person has a medical information sheet that contains (name, age, sex, family history, disease duration, first symptoms, smoking, diabetes, hypertension).

*Immunological study*

To measure the concentration of serum IL-1β, an Enzyme-Linked ImmunoSorbent Assay (ELISA) pack was used in this study, which is a quantitative sandwich enzyme immunoassay method. This is the antibody specific to interleukin 1β, already coated on a microplate. Standardization and samples when pipetted into the wells, the coated antibody binds to any present IL-1β. The collected IL-1β protein in a sample is detected by adding a biotin-conjugate antibody after an extensive wash. In order to create the signal, the horseradish peroxidase (HRP)-conjugated Streptavidin is applied first, followed by the addition of the tetramethyl-benzidine (TMB) reagent. After washing to dispose of any unbound mixture, an enzyme conjugate is added to the wells. To halt the color development and intensity, which are directly related to the amount of bound protein, a stop solution (sulfuric acid) is used. The amount of absorption was read at 450 nm when the reaction ended.

*Statistical Analysis*

The statistical package for the social sciences (application SPSS) version 21 was used to statistically evaluate all data. In this package, the Mann-Whitney U test was utilized to compare means between two groups, but the T-test was used for numerical variables that were presented as mean (SD) when the variables were distributed normally. Significant results were defined as those with a P-value less than 0.05.

## 3. Results and Discussion

*Comparison of serum IL-1β concentration in RA patients and control subjects.*

Table 1: Serum IL-1β levels in RA patients and controls.

| | Group | N. | Mean | ±SD | P.value |
|---|---|---|---|---|---|
| **IL-1β** | RA | 71 | 23.41 | 15.97 | 0.039* |
| | Control | 46 | 13.02 | 36.92 | |

SD: Standard deviation
*: Significant difference

The study found a significant increase (p=0.039) in IL-1β levels among RA patients (23.41± 15.97) compared to the control group (13.02± 36.92), as depicted in table (1). The result agreed with Jiang *et al*. (2023) who observed that RA patients had significantly greater levels of IL-1β than the control group (p=0.034). [11]. A Chinese study finally confirmed that RA patients had significantly greater levels of IL-1β than the control group (p=0.001) [12]. However, Kobayashi *et al*. (2010) could not find any evidence of IL-1β in people with RA [13]. This could be because they are taking very strong anti-inflammatory drugs.

**DOI: 10.36320/ajb/v16.i3.17234**

Genetic predisposition, activation of the immune system, and finally inflammatory signaling pathways all increase the IL-1β levels in individuals with RA [14]. In cases of RA, raised levels of IL-1β are connected to elevated levels of disease activity and progression of joint damage, causing severe pain, swelling, and stiffness. This is done through the stimulation of matrix metalloproteinase production and promotion of the formation of proinflammatory cytokines, where in the end it results in inflammation of the synovial membrane, thus causing synovitis, a characteristic feature of RA. Moreover, it can contribute to systemic signs of RA, such as fatigue, fever, and increased risk for cardiovascular diseases [15,16].

### *Clinical and sociodemographic factors in relation to the amount of IL-1β in serum among RA patients.*

The level of IL-1β didn't significantly differ in terms of sex, age, duration of RA, the first symptoms of RA in patients, the presence of hypertension, and diabetes ($p > 0.05$). In the meantime, the concentration of IL-1β (22.60±13.51) in patients with RA who smoked increased significantly in comparison with not smoking patients (14.90 ± 7.95) ($p = 0.045$). As shown in Table (2).

Table 2: The relationship between IL-1β and sociodemographic and clinical characteristics

| Clinical and Sociodemographic Features | | N | Mean | ±SD | P. Value |
|---|---|---|---|---|---|
| **Sex** | Female | 52 | 22.04 | 14.31 | 0.233 |
| | Male | 19 | 17.82 | 8.75 | |
| **Age** | >40 | 45 | 19.27 | 12.78 | 0.167 |
| | ≤40 | 26 | 23.76 | 13.50 | |
| **Duration** | >5 | 32 | 22.14 | 13.56 | 0.481 |
| | ≤5 | 39 | 19.91 | 12.86 | |
| **Family History** | Yes | 28 | 23.04 | 14.80 | 0.116 |
| | No | 43 | 18.09 | 8.81 | |
| **Smoking** | Yes | 14 | 22.60 | 13.51 | 0.045* |
| | No | 57 | 14.90 | 7.95 | |
| **First symptom** | Swollen + tender joints | 27 | 18.55 | 12.48 | 0.198 |
| | Morning stiffness | 44 | 22.64 | 13.10 | |
| **Hypertension** | No | 47 | 20.56 | 12.81 | 0.638 |
| | Yes | 24 | 22.11 | 13.40 | |
| **Diabetes Mellites** | No | 55 | 21.90 | 13.21 | 0.331 |
| | Yes | 16 | 18.29 | 11.90 | |

SD: Standard deviation
*: Significant difference

This result was compatible with Adachi and colleagues [17], who found significantly high levels of IL-1β has been detected in RA patients who smoke ($p= 0.01$) [17]. In patients with RA, smoking increasing the inflammatory process through increased oxidative stress, activation of pro-inflammatory pathways, and immune dysfunction by directly acting on the synovial tissue. Such pathways increase the levels of IL-1β, which contribute to RA worsening and progression in patients [18].

On the other hand, the current study revealed no correlation between IL-1β with clinical and sociodemographic features (age, sex, duration of RA, family history, first symptom, Hypertension, and Diabetes Mellites), these results agreed with the Japanese study that showed no association between IL-1β with these clinical and sociodemographic features [19]. This lack of association with clinical and sociodemographic features of RA patients could be the result of genetic variability in IL-1β expression, the complexity of the cytokine network in RA, and the very diverse impact of clinical and sociodemographic factors on immune responses. These factors obscure the direct association between IL-1β and some characteristics of the patient and emphasize multiple facets that must be used to understand the pathogenesis of RA [20,21].

## Conclusion

Generally, there was a clear and prominent relationship between serum IL-1β levels and RA in the Iraqi Arab population, especially smoker patients.

## Acknowledgment

I'd like to express my heartfelt thanks and thank all the staff of the Joints Centre of Al-Sader Hospital and Al-Amin Laboratory for their help in this project.

## Funding Information

Nil

## Ethics

This study was approved by the medical ethics committee of the University of Kufa (2017). Parents gave both written and verbal consent, and both participants and researchers agreed to the publishing of the results.

## References

1. Sabet, M. N., Nasrabadi, N., Jalili, Z., Pakzad, B., Davar, S., Ehtesham, N., Jafarpour, S., Mosallaei, M., & Esmaeilzadeh, E. (2021). Association of polymorphisms in the NLRP3 gene and rheumatoid arthritis in Iranian patients. *Iranian Journal of Immunology, 18*(3), 249–258. https://doi.org/10.22034/iji.2021.89507.1950

2. Ding, Q., Hu, W., Wang, R., Yang, Q., Zhu, M., Li, M., Cai, J., Rose, P., Mao, J., & Zhu, Y. Z. (2023). Signaling pathways in rheumatoid arthritis: Implications for targeted therapy. *Signal Transduction and Targeted Therapy, 8*(1). https://doi.org/10.1038/s41392-023-01331-9

3. Prasad, P., Verma, S., Surbhi, Ganguly, N. K., Chaturvedi, V., & Mittal, S. A. (2023). Rheumatoid arthritis: Advances in treatment strategies. *Molecular and Cellular Biochemistry, 478*(1), 69–88. https://doi.org/10.1007/s11010-022-04492-3

4. Su, J., Hu, W., Ding, Y., Zhang, P., Li, T., Liu, S., & Xing, L. (2024). Serum GM-CSF level is a predictor of treatment response to tocilizumab in rheumatoid arthritis patients: A prospective observational cohort study. *Arthritis Research & Therapy, 26*(1), 130. https://doi.org/10.1186/s13075-024-03373-y

5. Lopez-Castejon, G., & Brough, D. (2011). Understanding the mechanism of IL-1β secretion. *Cytokine & Growth Factor Reviews, 22*(4), 189–195. https://doi.org/10.1016/j.cytogfr.2011.10.001

6. Kondo, N., Kuroda, T., & Kobayashi, D. (2021). Cytokine networks in the pathogenesis of rheumatoid arthritis. *International Journal of Molecular Sciences, 22*(20). https://doi.org/10.3390/ijms222010922

7. Cronstein, B. N., & Sitkovsky, M. (2017). Adenosine and adenosine receptors in the pathogenesis and treatment of rheumatic diseases. *Nature Reviews Rheumatology, 13*(1), 41–51. https://doi.org/10.1038/nrrheum.2016.178

8. Weinblatt, M. E., Fleischmann, R., Huizinga, T. W. J., Emery, P., Pope, J., Massarotti, E. M., van Vollenhoven, R. F., Wollenhaupt, J., Bingham, C. O., Duncan, B., Goel, N., Davies, O. R., & Dougados, M. (2012). Efficacy and safety of certolizumab pegol in a broad population of patients with active rheumatoid arthritis: Results from the REALISTIC phase IIIb study. *Rheumatology, 51*(12), 2204–2214. https://doi.org/10.1093/rheumatology/kes150

9. Okano, T., Inui, K., Tada, M., Sugioka, Y., Mamoto, K., Wakitani, S., Koike, T., & Nakamura, H. (2016). Levels of interleukin-1 beta can predict response to tocilizumab therapy in rheumatoid arthritis: The PETITE (predictors of effectiveness of tocilizumab therapy) study. *Rheumatology International, 36*(3), 349–357. https://doi.org/10.1007/s00296-015-3379-x

10. Bedaiwi, M. K., Almaghlouth, I., & Omair, M. A. (2021). Effectiveness and adverse effects of anakinra in the treatment of rheumatoid arthritis: A

systematic review. *European Review for Medical and Pharmacological Sciences, 25*(24), 7833–7839. https://doi.org/10.26355/eurrev_202112_27630

11. Jiang, Q., Wang, X., Xu, X., Hu, L., Zhou, G., Liu, R., Yang, G., & Cui, D. (2023). Inflammasomes in rheumatoid arthritis: A pilot study. *BMC Rheumatology, 7*(1), 39. https://doi.org/10.1186/s41927-023-00353-8

12. Cheng, L., Liang, X., Qian, L., Luo, C., & Li, D. (2021). NLRP3 gene polymorphisms and expression in rheumatoid arthritis. *Experimental and Therapeutic Medicine, 22*(4), 1110. https://doi.org/10.3892/etm.2021.10544

13. Kobayashi, T., Yokoyama, T., Ishida, K., Abe, A., Yamamoto, K., & Yoshie, H. (2010). Serum cytokine and periodontal profiles in relation to disease activity of rheumatoid arthritis in Japanese adults. *Journal of Periodontology, 81*(5), 650–657. https://doi.org/10.1902/jop.2010.090688

14. Chen, P.-K., Tang, K.-T., & Chen, D.-Y. (2024). The NLRP3 inflammasome as a pathogenic player showing therapeutic potential in rheumatoid arthritis and its comorbidities: A narrative review. *International Journal of Molecular Sciences, 25*(1), 626. https://doi.org/10.3390/ijms25010626

15. Firestein, G. S., & McInnes, I. B. (2017). Immunopathogenesis of rheumatoid arthritis. *Immunity, 46*(2), 183–196. https://doi.org/10.1016/j.immuni.2017.02.006

16. Jang, S., Kwon, E. J., & Lee, J. J. (2022). Rheumatoid arthritis: Pathogenic roles of diverse immune cells. *International Journal of Molecular Sciences, 23*(2). https://doi.org/10.3390/ijms23020905

17. Adachi, M., Okamoto, S., Chujyo, S., Arakawa, T., Yokoyama, M., Yamada, K., Hayashi, A., Akita, K., Takeno, M., Itoh, S., Takii, T., Waguri-Nagaya, Y., Otsuka, T., Hayakawa, K., Miyazawa, K., & Onozaki, K. (2013). Cigarette smoke condensate extracts induce IL-1β production from rheumatoid arthritis patient-derived synoviocytes, but not osteoarthritis patient-derived synoviocytes, through aryl hydrocarbon receptor-dependent NF-κB activation and novel NF-κB sites. *Journal of Interferon and Cytokine Research, 33*(6), 297–307. https://doi.org/10.1089/jir.2012.0107

18. Gao, J., Zhang, H., Yang, Y., & Tao, J. (2023). Therapeutic potential of targeting the NLRP3 inflammasome in rheumatoid arthritis. *Inflammation, 46*(3), 835–852. https://doi.org/10.1007/s10753-023-01795-5

19. Shoda, H., Nagafuchi, Y., Tsuchida, Y., Sakurai, K., Sumitomo, S., Fujio, K., & Yamamoto, K. (2017). Increased serum concentrations of IL-1 beta, IL-21, and Th17 cells in overweight patients with rheumatoid arthritis. *Arthritis Research & Therapy, 19*(1), 111. https://doi.org/10.1186/s13075-017-1308-y

20. Dinarello, C. A. (2019). The IL-1 family of cytokines and receptors in rheumatic diseases. *Nature Reviews Rheumatology, 15*(10), 612–632.

https://doi.org/10.1038/s41584-019-0277-8

21. Arend, W. P., & Firestein, G. S. (2012). Pre-rheumatoid arthritis: Predisposition and transition to clinical synovitis. *Nature Reviews Rheumatology*, *8*(10), 573–586. https://doi.org/10.1038/nrrheum.2012.134