Research article

An assessment of serum interleukin - 39 in rheumatoid arthritis patients from Iraq

Wafaa Tialp Mohammed[1], Mohammed Hadi Munshed Alosami[2], Alia Essam Mahmood Alubadi[3]

[1,3]Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq
[2]College of Medicine, University of Baghdad, Baghdad, Iraq

(Received: June 2022 Revised: July 2022 Accepted: August 2022)

Corresponding author: **Wafaa Tialp Mohammed.** Email: talbw31@gmail.com

ABSTRACT

Introduction and Aim: The pro-inflammatory cytokine IL-39, a member of the IL-12 family plays a key role in the inflammatory response by modulating immune cell activity and inflammation. A literature search shows no study undertaken for the effect of IL-39's on arthritis so far. Hence, the purpose of this study was to investigate the role of IL-39 in rheumatoid arthritis.

Materials and Methods: This study involved 80 patients with rheumatoid arthritis registered at the Rheumatology Clinic at Baghdad teaching hospital. The patients were divided into three groups based on treatments received. Group 1 included patients who were not on any treatment for arthritis, Group 2 with patients on hydroxychloroquine and or prednisone treatment, and Group 3 that received Enbrel® (etanercept) and HUMIRA® (adalimumab**)** treatment for rheumatoid arthritis. A control group was included in the study. Patients in all groups were assessed for their serum IL-39 concentration, C - reactive protein, Anti-cyclic citrullinated peptide antibody (ACCP) and ESR.

Results: The patient age and BMI were not significantly different between the groups receiving treatment for Rheumatoid arthritis. A significant increase in the interleukin 39 concentration was observed in treatment groups (G1, G2, G3) as compared to normal healthy controls regardless of whether they were positive or negative for the anti-CCP test.

Conclusion: This study showed that the serum interleukin IL-39 levels significantly increased in patients diagnosed with rheumatoid arthritis thus suggesting that IL-39 could be considered as a potential inflammatory biomarker of RA.

Keywords: Rheumatoid arthritis; IL39; interleukin-12 family cytokines; Anti-cyclic citrullinated peptide antibody

INTRODUCTION

The pro-inflammatory cytokine interleukin 39 (IL-39) is a heterodimer and a member of the interleukin 12 family (1). IL-39 is secreted by many types of immune cells such as B cells, dendritic cells, and macrophages (2) and plays an essential role in inflammatory responses by organizing immune cell functions and inflammation (2,3). Interleukin-12 family cytokines including (interleukin 23) and (interleukin 12) have been shown in numerous studies to regulate the immune system, whereas (interleukin 35) and (interleukin 27) alleviate autoimmune disorders (4). Cytokine IL-39 was shown to play a role in the etiology of lupus erythematosus and psoriasis in mice (5) and the use of anti-IL-39 polyclonal antibodies effectively decreased the infiltration of inflammatory cells and autoantibody titers ameliorating the autoimmune symptoms (6).

Similarly, a study by Yang and his colleagues, showed that in patients suffering from the autoimmune neuromyelitis optical spectrum disorders, the disease severity to be associated to elevated serum levels of interleukin-39 when compared with healthy controls and non-inflammatory neurological disorders patients (3). A literature search on the role of IL-39 in patients suffering from rheumatoid arthritis revealed that no such study has been undertaken so far. Hence in this study, we aimed to investigate the role of IL-39 in rheumatoid arthritis, which is an autoimmune illness.

MATERIALS AND METHODS

This study included 80 patients with rheumatoid arthritis registered at the Rheumatology Clinic of the teaching hospital in Baghdad. Patients were diagnosed based on their symptoms and classified as having rheumatoid arthritis according to the criteria established by the American College of Rheumatology (ACR) and the 2010 European Society of Rheumatology (ACR/European League against Rheumatology) (7)

The patients were divided into three groups based on the type of treatment they received. Group 1 (n=16) included patients who had rheumatoid arthritis but not on any treatment. The treatment groups, Group 2 (n=26) included patients under non-biological treatment with hydroxychloroquine and/or prednisone and Group 3 (n=38) under biological treatment with

Enbrel® (etanercept) and HUMIRA® (adalimumab) drugs as treatment options for rheumatoid arthritis.

The inclusion criteria for patients in Groups 1-3 included only patients who suffered from rheumatoid disease, but were free of any infectious, immune, or chronic diseases, suffering from organ failures and not vaccinated for 6 months. The study included a control group G4 (n=18) which included subjects who were free from infection and rheumatoid arthritis and negative for RF, anti-CCP and CRP. The study participants were checked for their BMI based on their body height (meter) and bodyweight (kilogram) and using the formula: BMI = kg/m^2. Blood sample was collected from each of the participant and the serum subjected to qualitative and semi-quantitative detection of C – Reactive Proteins and rheumatoid factor (RF) using the respective CRP-latex and RF-latex slide agglutination test (Agappe Diagnostics Switzerland GmbH). The serum was subjected to semi-quantitative detection of anti-CCP antibody using commercially available kits (MyBioSource/ USA), and human antibody concentration by ELISA (indirect ELISA). The IL-39 concentration in serum was measured using a commercial IL39 ELISA kit (MyBioSource, USA) following the manufacturer's instructions.

Statistical analysis

For numerical variables, the mean and standard error (SE) were calculated using SPSS 20 analysis of variance (ANOVA), and for categorical variables, the percentage was calculated using SPSS 20 (chi-square tests of goodness of fit and independence were used). The differences between the averages of groups were evaluated by least significant difference –LSD test at a significant level of 0.01 and 0.05. The letters A, B, C and D express the significance for column while a, b, c, and d express the significance for rows. All data showed normal distribution by using the Shapiro-Wilk test.

RESULTS

Eighty blood samples of registered patients were collected from the Rheumatology Consulting Clinic/Baghdad Teaching Hospital for diagnosis and treatment. The patients were divided into Groups 1-3 based on the nature of their treatment options. The demographic features of the participants, the laboratory tests undertaken to diagnose rheumatoid disease, such as the rheumatoid factor test (RF), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and anti-CCP level is presented in Table 1.

In this study the patient age and BMI were observed to be not significantly different between the treatment groups (G1, G2, G3) as well as control (G4) group (Table 1). Among the groups, the percentage of patients positive for the C-reactive protein (CRP) was highest in Groups 2 and 3. A high significance ($p < 0.01$) was observed for CRP among the three groups (G1-G3) as compared to the control group. Patients positive for the Rheumatoid Factor (RF), were highest in Group 1 that included patients diagnosed as having rheumatoid arthritis, and not on any treatment. This was followed by Groups G3 and G2.

In comparison with the control group, the number of patients positive for the anti-CCP (anti-cyclic citrullinated peptide) test was significantly higher in the G1, G2, and G3 groups (Table 1). An elevated level of ESR and IL-39 was observed in patients with rheumatoid arthritis in all the groups as compared to control group with no arthritis which was found to be statistically significant.

Table 1: Demographic features of participants included in the study

Parameter	RA patient groups			Control G4 (n=18)	P value
	G1 (n=16)	G2 (n=26)	G3 (n=38)		
Age	40.3±3.2	46.1±2.2	43.8±2.3	46.8±2.9	NS
BMI	21.2±0.5	22.8±0.5	21.3±0.4	26.7±0.7	NS
Anti-CCP	0.48±0.09	0.23±0.05	0.34±0.05	0.12±0.004	0.009**
ESR	41.1±6.1	41.3±3.5	40.2±4.4	15.2±1.2	0.0004**
IL-39	105.1±12.7	113.58±8.9	116.6±6,6	49.5±3.7	0.00001**
CRP +ve (%)	10 (62.5%)	16 (61.5%)	19 (50%)	0 (0%)	0.0002** (Chi square= 19.86)
RF +ve (%)	8 (50%)	7 (26.9%)	17 (44.7%)	0 (0%)	0.004** (Chi square =13.83)

RA: Rheumatoid arthritis; Control group (G4); Anti-CCP: Anti-cyclic citrullinated peptide antibody, ESR: Erythrocyte Sedimentation Rate, CRP: C-Reactive Protein; RF: Rheumatoid Factor. P value < 0.05* is significant while P value < 0.01** is highly significant and NS not significant.

Table 2: The serum levels of IL-39 levels based on anti-CCP (ACCP)

Group	**IL-39 for tested groups Mean ± SE**		
Control	**G4**	**G4**	**G4**
P value	49.5±3.8 C	49.5±3.8 C	49.5±3.8 C
Negative ACCP	**G1**	**G2**	**G3**
0.001	113.2±8.9 B	89.5±7.4 B C	130.4±28.9 A a
Positive ACCP	**G1**	**G2**	**G3**
0.001	119.9±10.3 B	137.6 ± 26.5 A	79.8±19.2 B c
P value 1	0.001	0.001	0.001
P value 2	NS	0.001	0.001

Data indicated as mean ± SE. LSD test with significant level of 0.01 and 0.05 and the letters A, B, C, and D express significance for column while a, b, c and d express the significance for rows, G1 (RA+ no treatment), G2 (RA+ treatment with non-biological drugs), G3 (treatment with biological drugs) and G4 (control group). P value 1: Between patients' groups and control. P value 2: Between patients' groups only

The serum IL-39 levels based on anti-CCP test are presented in Table 2. The three pathological groups (G1-G3) were divided according to the anti-CCP test results as negative and positive groups. A significant difference for the anti-CCP values was seen between the positive and negative group. Among Group G1 no significant difference for IL-39 was observed between the anti-CCP positive and negative groups. However, Interleukin IL-39 levels were found to be significantly higher in group G2 in the anti-CCP positive group, and group G3 in anti-CCP negative group (Table 2). The study indicated an increase in serum IL-39 levels in treatment groups (G1, G2, G3) as compared to controls (G4) regardless of whether they were positive or negative for the anti-CCP test.

DISCUSSION

Rheumatoid arthritis (RA) is a chronic autoimmune disease responsible for inflammation of the joints. The serological markers for RA are rheumatoid factors (a class of immunoglobulins) and antibodies to citrullinated proteins (8,9). Therefore, diagnoses of RA in patients have been based on tests that allow detection of rheumatoid factors and for antibodies recognizing cyclic citrullinated peptides (ACCP) in serum (9). Although the detection of rheumatoid factors has been proven to be specific for RA in patients, studies have also indicated the presence of these factors in non-rheumatic conditions such as infections and chronic diseases (10) and healthy individuals irrespective of gender within a population (11,12) and hence the RF level to monitor RA disease is not recommended. Recent studies have demonstrated that the ACCP test is highly specific for the detection of RA (13,14) or a combination of ACCP and RF test being more sensitive than either test alone (15).

In this study, the prevalence of rheumatoid factor as well as the anti-CCP levels was seen to be highest in patients with RA and no treatment group in comparison to treatment groups (G2 and G3) wherein, there was a decrease in these two test levels. Our results are in accordance with previous studies (16) which indicated that there is an association between a decrease in RF and ACCP levels with the concomitant use of drugs used in RA treatments.

The interleulin-12 cytokine IL-39 is known to mediate inflammatory response and is implicated in immunopathogenesis of diseases such as psoriasis (17), systemic lupus erythematosus (4), myocardial infarction (18) and hepatocyte necrosis (19). This study indicated an increased level of IL-39 in RA patients (G1-G3) compared to healthy control assuming significance as results show that IL-39 probably contributes to inflammatory response in RA as seen in other disease studied.

Furthermore, in RA proinflammatory cytokines are known to activate the JAK/STAT signal transduction pathway to mediate an inflammatory response (20). In systemic lupus erythematosus disease, the cytokine IL-39 secreted by activated B-cells has been shown to mediate its effect through the activation of STAT1/ STAT3 of the signal transduction pathway (5). This shows IL-39 being linked to common immune processes and thus we assume that in RA the IL-39 may be playing a key role in contributing to the immunopathogenic mechanisms, mediating an inflammatory response. Our results also show that in patients with RA, IL-39 levels could be used as a possible target for diagnosis of the disease.

Enbrel (etanercept) and Humira (adalimumab) are tumour necrosis factor (TNF) inhibitors used to treat rheumatoid arthritis and juvenile idiopathic arthritis (21, 22) while hydroxychloroquine and prednisone

drugs are prescribed in the management of rheumatoid arthritis (23, 24). Systemic blocking of TNF, a proinflammatory cytokine has been shown to block and control the inflammatory process of RA (25, 26) the results observed in this study for serum IL-39 levels based on RA antibody ACCP (Table 2) are in line with previous studies and showed that patients treated with TNF inhibitors exhibit significant interaction effects in response to anti-TNF therapy for rheumatoid arthritis.

CONCLUSION

Serum IL-39 levels were considerably elevated in rheumatoid arthritis (RA) patients, suggesting that IL-39 may be a potential inflammatory biomarker of RA.

ACKNOWLEDGEMENT

We thank Mustansiriyah University, Baghdad, Iraq for the support in carrying out this work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Lu, Z., Xu, K., Wang, X., Li, Y.,Li, M., Interleukin 39: a new member of interleukin 12 family. Eur J Immunol. 2020; 45(2):214-217.
2. Wang, X., Liu, X., Zhang, Y., Wang, Z., Zhu, G., Han, G., *et al.*, Interleukin (IL)-39 [IL-23p19/Epstein–Barr virus-induced 3 (Ebi3)] induces differentiation/expansion of neutrophils in lupus-prone mice. Clin Exp Immunol. 2016;186(2):144-156.
3. Yang, M.G., Tian, S., Zhang, Q., Han, J., Liu, C., Zhou, Y., *et al.*, Elevated serum interleukin-39 levels in patients with neuromyelitis optica spectrum disorders correlated with disease severity. Mult Scler Relat Disor. 2020; 46:102430.
4. Ecoeur, F., Weiss, J., Schleeger, S., Guntermann, C. Lack of evidence for expression and function of IL-39 in human immune cells. Plos one, 2020;15(12):0242329.
5. Wang, X., Wei, Y., Xiao, H., Liu, X., Zhang, Y., Han, G., *et al.*, A novel IL-23p19/Ebi3 (IL-39) cytokine mediates inflammation in Lupus-like mice. Eur J Immunol .2016; 46(6):1343-1350.
6. Wang, X., Zhang, Y., Wang, Z., Liu, X., Zhu, G., Han, G., et al., Anti-IL-39 (IL-23p19/Ebi3) polyclonal antibodies ameliorate autoimmune symptoms in lupus-like mice. Mol Med Rep. 2018; 17(1):1660-1666.
7. Taylor, P. C. Update on the diagnosis and management of early rheumatoid arthritis. Clinical medicine (London). 2020; 20(6): 561-564.
8. Bobbio-Pallavicini F, Caporali R, Alpini C, Moratti R, Montecucco C. Predictive value of antibodies to citrullinated peptides and rheumatoid factors in anti-TNF-alpha treated patients. Ann N Y Acad Sci. 2007;1109: 287-295.
9. De Rycke L, Peene I, Hoffman IE, Kruithof E, Union A, Meheus L, *et al.*, Rheumatoid factor and anticitrullinated protein antibodies in rheumatoid arthritis: diagnostic value, associations with radiological progression rate, and extra-articular manifestations. Ann Rheum Dis. 2004; 63(12):1587-1593.
10. Wu, C.Y., Yang, H.Y., Luo, S.F., Lai, J.H. From rheumatoid factor to anti-citrullinated protein antibodies and anti-carbamylated protein antibodies for diagnosis and prognosis prediction in patients with rheumatoid arthritis. Int J Mol Sci., 2021; 22(2):686.
11. Tasliyurt, T., Kisacik, B., Kaya, S.U., Yildirim, B., Pehlivan, Y., Kutluturk, F., *et al.*, The frequency of antibodies against cyclic citrullinated peptides and rheumatoid factor in healthy population: a field study of rheumatoid arthritis from northern Turkey. Rheumatol Int. 2013; 33(4):939-942.
12. Korpilähde, T., Heliövaara, M., Kaipiainen-Seppänen, O., Knekt, P., Aho, K. Regional differences in Finland in the prevalence of rheumatoid factor in the presence and absence of arthritis. Ann Rheum Dis. 2003;62(4):353-355.
13. Vander Cruyssen B., Peene I., Cantaert T., Hoffman IE., De Rycke L., Veys EM. *et al*., Anti-citrullinated protein/peptide antibodies (ACPA) in rheumatoid arthiritis: specificity and relation with rheumatoid factor. Autoimmun Rev. 2005; 4(7):468-474.
14. Nishimura, K., Sugiyama, D., Kogata, Y., Tsuji, G., Nakazawa, T., Kawano, S., *et al.*, Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. Ann Intern Med. 2007 Jun 05;146(11):797-808.
15. Ingegnoli F, Castelli R, Gualtierotti R. Rheumatoid factors: clinical applications. Dis Markers. 2013;35(6):727-734.
16. Kao, C.M., Yen, T.H., Lai, K.L., Chen, Y.M., Huang, W.N., Chen, Y.H. Association between a decrease in anti-cyclic citrullinated protein levels with the concomitant use of disease-modifying antirheumatic drugs in rheumatoid arthritis patients treated with tumor necrosis factor inhibitors or abatacept. Formosan J Rheumatol. 2021, 35:47-56.
17. Tachibana, K., Tang, N., Urakami, H., Kajita, A., Kobashi, M., Nomura, H., *et al.*, Multifaceted Analysis of IL-23A- and/or EBI3-Including Cytokines Produced by Psoriatic Keratinocytes. Int J Mol Sci. 2021; 22(23):12659.
18. Luo, Y., Liu, F., Liu, H., Chen, H., Cheng, W., Dong, S., Xiong, W. Elevated serum IL-39 in patients with ST-segment elevation myocardial infarction was related with left ventricular systolic dysfunction. Biomark Med .2017; 11(6): 419-426.
19. Li, Y., Gong, L., Weng, L., Pan, X., Liu, C., Li, M. Interleukin-39 exacerbates concanavalin A-induced liver injury. Immunopharmacol Immunotoxicol. 2021; 43(1): 94-99.
20. Malemud, C.J. The role of the JAK/STAT signal pathway in rheumatoid arthritis. Ther Adv Musculoskelet Dis. 2018; 10(5-6): 117-127.
21. Kim, E.S., Garnock-Jones, K.P., Keam, S.J. Adalimumab: a review in hidradenitis suppurativa. Am J Clin Dermatol. 2016; 17(5): 545-552.
22. Zhao, S., Mysler, E., Moots,R. J. Etanercept for the treatment of rheumatoid arthritis. Immunotherapy, 2018;10(6):433-445.
23. Krasselt, M., Baerwald, C. Efficacy and safety of modified-release prednisone in patients with rheumatoid arthritis. Drug Des Devel Ther. 2016; 10:1047.
24. Schrezenmeier, E., Dörner, T. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. Nat Rev Rheumatol. 2020; 16(3):155-166.
25. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011; 365 (23):2205–2219.
26. Julià, A., López-Lasanta, M., Blanco, F., Gómez, A., Haro, I., Mas, A.J., *et al.*, Interactions between rheumatoid arthritis antibodies are associated with the response to anti-tumor necrosis factor therapy. BMC Musculoskelet. Disord. 2021; 22(1):1-7.