Study of some immunological indicators of interleukin-9 in Rheumatoid arthritis of Iraqi Patients

Ibtesam B. Hassan*, Aseel J. Kadim, Ammar A. Sultan
Biology Department, College of Education for Pure Science, The University of Diyala, Iraq.

ibtesambh67@gmail.com

Abstract. Rheumatoid arthritis is a chronic disease of the joints and passes through alternating stages of healing and relapse as it has genetic, clinical, pathological, and immunological characteristics. The present study was designed to evaluate the immunological status of some Iraqi patients with rheumatoid arthritis. The present study was conducted on a group of patients reviewed at Baquba Teaching Hospital / Consultation Clinic and from all districts of Diyala Province. For the period 2018-7-20 until 2019-2-1, Blood samples were collected from 50 patients with rheumatoid arthritis aged 29-75 years. On the other hand, 50 other blood samples from healthy individuals were collected as a control group, aged between 25 and 75 years. The study involved investigated the relationship between the immunological detection of the cellular motility of the IL-9 using ELIZA technique. The results of the present study showed that the percentage of female infection with rheumatoid arthritis was 94% higher than that of males by 6%. Also, the results recorded the highest incidence of rheumatoid arthritis in the 45-54 age group at the main of (369.12 ± 7.75) pg / ml and 38%, While the lowest recorded cases of the disease in the age group 65-75 years at the main (350.40 ± 10.73) pg / ml and by 16%. As, the results of the present study showed a significant increase in the level of the concentration of interleukin 9 in the serum of patients with rheumatoid arthritis (362.29 ± 26.86 pg / ml) compared to control group (332.45 ± 27.58) pg / ml at a probability level (P <0.01) Indicates a relationship between interleukin 9 and rheumatoid arthritis.

Keywords. ELIZA ,IL-9, Rheumatoid arthritis.

1. Introduction
Rheumatoid arthritis (RA) is the most common autoimmune disease in the world [1]. Rheumatoid arthritis is a primary clinical manifestation that mainly affects small joints in the hands and feet [2]. Arthritis may spread to the body, leading to swelling, stiffness and joint damage with the possibility of loss of function [3]. It was recorded in 1990, about 28,000 deaths worldwide [4]. In 2010, resulting in about 49,000 deaths worldwide [5]. In 2013, it resulted in approximately 38,000 deaths, and more than 24.5 million people developed rheumatoid arthritis in 2015. The proportion of rheumatoid arthritis in adults ranges from about 1-0.5% in developed countries [6]. T and B cells are mostly involved in the pathogenesis of RA along with the pro-inflammatory cytokines reaction such as TNF-α, IL-6, IL-1β -
PsA is a chronic inflammatory disease that affects the spine or peripheral joints of patients with psoriasis with a family history [8]. Long ago, PsA as a disease Th1 by IFN-γ, IL-12 signal cytokines [9]. IL-9 plays a crucial role in inflammatory diseases such as asthma, atopic dermatitis, systemic lupus erythematosus and rheumatoid arthritis, IL-9, the end of the 1980s, was first described as a growth factor; it is also a multifunctional cellular cytokines of the immune system and is still unknown or unknown cellular cytokines [10]. It has often been associated with allergies [11, 12]. The human IL-9 gene located on chromosome 5 (Mock et al., 1990) is a group of cytokine genes [13], produced by many cell types including helper T cells [14]. It was observed that IL-9 levels were higher in synovial fluid for RA disease and that IL-9 promotes T cell activity and proliferation in patients with rheumatoid arthritis [15]. For this reason, this study investigated the concentration of interleukin-9 in the serum of Iraqi patients with rheumatoid arthritis.

2. Materials and Methods

2.1. Subjects
The present study was conducted on a group of patients in Baquba Teaching Hospital / Consultative Clinic. Blood samples were obtained for patients with rheumatoid arthritis from all districts of Diyala province. Samples collection for the period from 20/07/2018 until 01/02/2019 and the number was a sample (100), has been diagnosed all cases by doctors jurisdiction of where the study included 50 medical condition and the number of males in which 3 and 6%, and the number of females 47 and by 94 And 50 blood samples were apparently collected. The number of males was 13 (26%), the number of females (37%) and 74% was used as a control group. and the ages ranged between (25 - 75) years, (3m) of intravenous blood was withdrawn by plastic medical syringes and blood was placed in test tubes for the purpose of separating the serum of them later "and conducting immunological tests on them. The level of cellular kinetics was quantified by using the sandwich ELISA test for 88 samples (44) patients and 44 healthy subjects according to the instructions provided by Elabscience.

2.2. Test Principle
This test used a number of ready-made enzymes according to the Immuno assay technique where the enzyme-linked immunoassay test (Sandwich ELISA test) was used to estimate the level of IL-9. Incubated and after the incubation process, anti-IL-9 antibodies marked with biotin were added to associate with HRP, which leads to the formation of the immune complex. After incubation, the unrelated enzymes were removed and the dish was washed and the base material was added, thus the solution was converted from blue to yellow with acid effect.

3. Results and Discussions

3.1. Percentages of IL-9 in the Study Group According to Age Groups
When comparing the level of IL-9 with age groups, it was found that the highest level of IL-9 was in the age group (45-54) years and was (369.12 ± 7.75) and the lowest level in the age group (65-75) years was (350.40 ± 10.73) with The absence of statistically significant difference as in Table (1).

| Patients Groups | N  | M ± Std. Error of Mean | p value |
|-----------------|----|------------------------|---------|
| 25 - 34         | 3  | 351.73±0.93            | N.S.    |
| 35 - 44         | 9  | 359.64±8.23            | N.S.    |
| 45 - 54         | 17 | 369.12±7.75            | N.S.    |
| 55 - 64         | 10 | 362.16±7.87            | N.S.    |
| 65 - 75         | 5  | 350.40±10.73           | N.S.    |
| Total           | 44 | 362.29±4.04            | N.S.    |
These results are consistent with those of Boots et al. [16] that show an increase in IL-9 concentration levels in rheumatoid arthritis in elderly people. A recent study found a link between decreased immune function and age as a major cause of rheumatoid arthritis [17].

3.2. Distribution of Study Groups and Percentages for Gender
The current study included 50 patients with rheumatoid arthritis between the ages of (25-75) years, where the number of males 3 and by 6%, the number of females was 47 and 94%, while the control group included 50 people who were apparently healthy and the number of males 13 by 26%, the number of females 37 by 74% under probability (P = 0.01) as shown in Table (2). These results indicate that the number of females infected with the disease more than the number of males. These results are identical to several studies that indicated that the incidence of the disease is greater in women compared to men. There are many possible causes of female tendency to be infected, including breastfeeding and use of contraceptives and also a short fertility period, and hormonal factors as a result of menoopause, where a lack of estrogen, this leads to a lack of calcium absorption and osteoporosis, which leads women to take progesterone and estrogen [18]. The current study also coincided with this study, which concluded that high autoimmune response leads to a higher rate of rheumatoid arthritis among females than males due to hormonal differences between them. Their effects on the immune response make these women more susceptible to autoimmune response as a result of activation of helper T cells that play a role in the development of infections and thus cause rheumatoid arthritis [19].

| Arithmetic mean ± standard deviation | Females | Males | the groups |
|-------------------------------------|---------|-------|------------|
| total summation % number % number   |         |       |            |
| 51.23±11.264 50 94 47 6 3 Patients group |         |       |            |
| 45.16±9.19 50 74 37 26 13 Control group |         |       |            |

These results agreed with the study carried out by [20] as it showed that the prevalence of rheumatoid arthritis disease rheumatoid among women more than males as 82.9% total, while men 17.1% this is what the [21] as the results of their study showed that the prevalence of Rheumatoid arthritis among women is the highest proportion of men (85.0%). It also agreed with Jasim's findings [21], as the results of his study showed that the prevalence of rheumatoid arthritis among women is higher than men with 74.19% for women and 9.68% for men.

3.3. Measure the level of IL-9 in serum
The current study is the first in Iraq, was measured at the Interleukin- 9 in the serum of 44 patients from patients with rheumatoid arthritis group and compare it with the serum of 44 people from the control group. Results shown in the table (1). Patients were significantly (362.29 ± 26.86) pg / ml, compared to the control group which recorded a significant decrease in the level of IL-9 (332.45 ± 27.58) pg / ml, and under probability (P <0.01).
Above results indicate a clear height between interleukin-9 in patients group with rheumatoid arthritis disease, suggesting that autoimmune disease is linked to multiple genes [23]. Studies have shown that the multifunctional IL-9 may be involved with Th9 cells in several types of inflammatory diseases. These findings are consistent with our findings suggesting that IL-9 and Th9 cells may significantly contribute to increased immune responses in inflammatory joints of rheumatoid arthritis [24]. Genes with HLA-DRB1 genes, especially HLA-DRB 04 allele, which plays an important role in the development of rheumatoid arthritis in Iraqi patients [21]. The current study agrees with the study that the strong relationship between elevated IL-9 and autoimmune concentrations has highlighted its potential role in T cell-based B cell differentiation, expansion and antibody production in rheumatoid arthritis patients [25].

4. Conclusions

1. The results of the present study showed that the percentage of females with rheumatoid arthritis is higher than that of males, 96% for females and 6% for males.
2. The age group 54-45 years is the most affected by rheumatoid arthritis by 38%, while the age group 25-34 was the lowest in the disease by 6%.
3. The results of the study showed an increase in the level of IL-9 in serum of patients with rheumatoid arthritis compared to the control group, indicating the role of IL-9 in arthritis processes.

References

[1] McInnes, L.B. and Schett, G.(2011). The pathogenesis of Rheumatoid Arthritis. N. Engl. J. Med. 365:2205-2219.
[2] Widdifield, J.; Paterson, J. M.; Bernatsky, S.; Tu, K.; Thorne, J. C.; Ahluwalia, V.; Ivers, N.; Butt, D.; Jaakkimainen, L.; Tomlinson, G. and Bombardier, C. (2013). The rising burden of rheumatoid arthritis surpasses rheumatology supply in Ontario. Can. J. Pub. Health. 104:450-455.
[3] Lee, S.; Boyle, D.; Berdeja, A. and Firestein, G. (2012). Regulation of inflammatory arthritis by the upstream kinase mitogen activated protein kinase 7 in the C-jun N-terminal kinase pathway. Arthritis research and therapy. 5: 233-243.
[4] Paget, S. A.; Lockshin, M. D. and Loebl, S. (2002). The Hospital for Special Surgery Rheumatoid Arthritis Handbook Everything You Need to Lozano, R.; Naghavi, M; Foreman, K; Lim, S; Shibuya, K; Aboyans, V.; Abraham, J; Adair, T; Abraham, J.; Adair, T.; et al. (2012). "Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010". Lancet. 380(9859):2095–128.
[5] Lozano R1, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D. et al., ( 2012). Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. Dec 15;380(9859):2095-128.
[6] Smolen, J. S.; Aletaha, D. and McInnes, I. B. (2016). "Rheumatoid arthritis" Lancet. 388 (10055): 2023–2038.
[7] Van de Sande, M.G. and Baeten, D.L.(2016). Immunopathology of synovitis: from histology to molecular pathways. Rheumatology, (Oxf) ; 55:599–606.

[8] Oliueri, I.; D’Angelo, S.; Palazzi, C. and Padula ,A.(2014). Advances in the management of psoriatic arthritis. Nat. Rev. Rheumatol. ; 10: 531–42.

[9] Barnas, J.L. and Ritchlin, C.T.(2015). Etiology and pathogenesis of psoriatic arthritis. Rheum. Dis. Clin. North. Am.; 41:643–63.

[10] Goswami, R. and Kaplan, M.H.(2011). A brief history of IL-9. J. Immunol. ,186:3283–8.

[11] Temann, U. A.; Geba, G. P.; Rankin, J. A. and Flavell, R. A.(1998). “Expression of interleukin 9 in the lungs of transgenic mice causes airway inflammation, mast cell hyperplasia, and bronchial hyperresponsiveness,” The Journal of Experimental Medicine, vol. 188, no. 7, pp. 1307–1320.

[12] Nicolaides, N.C. ; Holroyd, K.J. ; Ewart, S.L. ; Eiff, S.M. ; Kiser, M.B. ; Dragwa, C.R. ; Sullivan, C.D. ; Grasso, L. ; Zhang, L.Y. ; Messler, C.J. ; Zhou, T. ; Kleeberger, S.R. ; Buetow, K.H. ; Levitt, R.C. (1997). Interleukin 9: a candidate gene for asthma, Proc. Natl. Acad. Sci. U. S. A. 94(24) 13175–13180.

[13] Raychaudhuri, S.K.; Saxena, A. and Raychaudhuri, S.P.(2015). Role of IL-17 in the pathogenesis of psoriatic arthritis and axial spondylo arthritis. Clin. Rheumatol. ; 34:1019–23.

[14] Boots, A. M. H.; Maier, A. B.; Stinissen, P.; Masson, P.; Lories, R. J. and De Keyser, F. (2013). The influence of ageing on the development and management of rheumatoid arthritis. Nature Reviews Rheumatology, 9(10) , 604–613.

[15] Van Onna, M. and Boonen, A. (2016). The challenging interplay between rheumatoid arthritis, ageing and comorbidities. BMC Musculoskeletal Disorders, 17(1).

[16] Krishnan, E.; Sokka, T. and Hannonen, P. (2003). Smoking–gender interaction and risk for rheumatoid arthritis. Arth. Res. Ther. 5:158-162. Know. New York: John Wiley & Sons. p. 32. from the original on 2017-02-22.

[17] Kindt, T.; Goldspy, R. and Osborne, B. (2007). Rheumatoid Arthritis. In: Kuby Immunology. 6th ed. WH Freeman and company. New York. PP: 401-21.

[18] Jasim, N. Salah. (2016). Detection of Epstein Barr Virus in a sample of rheumatoid arthritis. Master Thesis, College of Science - Baghdad University, Iraq: 53-91.

[19] Hughes-Austin, J.M. ; Deane, K.D. ; Derber, L.A. ; Kolfenbach, J.R. ; Serbe, G.O. ; Sokolove, J.; Lahey, J.L. ; Weisman, M.H. ; Buckner, T.R. ; Mikuls, J.H. ; O’Dell, J.R.; et al.(2013).Multiple cytokines and chemokines are associated with rheumatoid arthritis-related autoimmunity in first-degree relatives without rheumatoid arthritis: Studies of the Aetiology of Rheumatoid Arthritis(SERA), Ann. Rheum. Dis. 72 (6) 901–907.

[20] Dantas, A.T. ; Marques, C.D. ; da Rocha Junior, L.F.; Cavalcanti, M.B.; Goncalves, S.M.; Cardoso, P.R.; Mariz Hde, A.; Rego, M.J.; Duarte, A.L.; Pitta, I.da. and Pitta, M.G.(2015). Increased serum interleukin-9 levels in rheumatoid arthritis and systemic lupus erythematosus: pathogenic Role or just an epiphenomenon. Dis. Markers,51963-8.