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Immunological Evaluation of Patients with Rheumatoid Arthritis in Baghdad Governorate

Maryam Hekmat Abdulateef [1*], Ibrahim Hadi Mohammed [2]

[1,2]*Department of Biology, College of Science, University of Diyala, Diyala City, Iraq.*

[*]Corresponding author: maryamhikmat1991@gmail.com

Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that primarily affects the joints. Inflammation of the synovium, cartilage, and bone erosion are the most prominent symptoms of RA. The stages of development of this disease are very complex, as synovial cells rupture and form inflammation of the synovium, followed by deterioration of cartilage and bone. Cytokines are proteins that play a crucial role in the onset and progression of disease. This study was conducted at Medical City Hospital in Baghdad. It included 50 blood samples from patients with rheumatoid arthritis (25 males and 25 females), in addition to 30 healthy people (15 males and 15 females) a control group, aged between 10 and 60 years. The levels of Interleukin-2 (IL-2) and Interleukin-6 (IL-6) were measured using enzyme-linked immunosorbent assay (ELISA), and Hemoglobin Test (Hb) , White blood cells (WBC) , Erythrocyte sedimentation rate (ESR) , Urea, , sugar test , Lactate dehydrogenase (LDH) and Creatinine were measured for all infected and healthy people. The results showed a significant increase ($P<0.05$) in all measurements of IL-2, IL-6, Hb,WBC, ESR, urea, , sugar, LDH and Creatinine .

Keywords: Rheumatoid Arthritis, Erythrocyte sedimentation rate , Interleukin-2 , Interleukin-6 , enzyme – linked immunosorbent assay

التقييم المناعي لمرضى التهاب المفاصل الروماتويدي في محافظة بغداد

مريم حكمت عبداللطيف[1*] , ابراهيم هادي محمد [2*]

[1,2] قسم علوم الحياة , كلية العلوم , جامعة ديالى , محافظة ديالى , العراق

الخلاصة

التهاب المفاصل الروماتويدي (RA) هو أحد أمراض المناعة الذاتية الالتهابية المزمنة التي تؤثر على المفاصل بشكل أساسي. يعد التهاب الغشاء المفصلي والغضاريف وتآكل العظام من أبرز أعراض مرض (RA). مراحل تطور هذا المرض معقدة للغاية، حيث تتمزق الخلايا الزليلية وتشكل التهابًا في الغشاء الزليلي، يليها تدهور الغضروف والعظام. السيتوكينات هي بروتينات تلعب دورًا حاسمًا في بداية ظهور المرض وتطوره. أجريت هذه الدراسة في مستشفى مدينة الطب ببغداد. شملت 50 عينة دم لمرضى مصابين بالتهاب المفاصل الروماتويدي (25 ذكرًا و 25 أنثى) بالاضافة الى 30 اصحاء (15 ذكرًا و 15 أنثى) مجموعة ضابطة تتراوح أعمارهم بين 10 إلى 60 عامًا. تم قياس مستوى IL-2 و IL-6 باستخدام مقياس الممتز المناعي المرتبط بالانزيم (ELISA) وقياس Urea , ESR , WBC واختبار السكر لجميع المصابين والاصحاء . وأظهرت النتائج زيادة معنوية ($P<0.05$) في كل قياسات IL-2 , IL-6 , WBC , ESR , اليوريا و السكر .

الكلمات المفتاحية : التهاب المفاصل الروماتويدي , سرعة ترسيب خلايا الدم الحمراء , انترلوكين -2 , انترلوكين – 6 , مقياس الممتز المناعي المرتبط بالانزيم.

1. Introduction

Rheumatoid arthritis (RA) is pathologically manifested as immune cell infiltration, hyperplasia of the synovial lining, pannus formation, and destruction of articular cartilage and bone is classified as a systemic poly-articular chronic autoimmune joint disease that primarily affects body joints [1]. Although the exact etiology of RA is unclear, it is certain that genetic and environmental factors have influences on RA occurrence. At present, RA affects approximately 0.5% to 1.0% of the population worldwide [2], and in particular, females are at higher risk of the disease (two to three times than males) [3]. RA patients typically experience morning stiffness. If left untreated, they could appear small focal necrosis, adhesion of granulation, and fibrous tissue on the articular surface, which lead to progressive joint ankylosis, destruction, deformities, and disability [4].

Cytokines control a wide range of inflammatory processes that are involved in the pathogenesis of rheumatoid arthritis (RA), a chronic inflammatory disease that predominantly involves the joints. An autocrine and paracrine cascade network communication through interleukins (ILs) has a pivotal role in the development of synovitis and perpetuation of the disease. IL-1 and IL-6 are among the most important ILs involved in RA [5]. Anakinra, a recombinant form of the naturally occurring interleukin-1 receptor antagonist (IL-1ra) plus methotrexate was proved to be effective in reducing the signs and symptoms of the disease in patients with inadequate responses to methotrexate alone [6]. This was also the case for a number of biologic agents that blocked the receptor of IL-6 [7].

The severity of illness and pain are closely associated with inflammation and oxidative stress [8]. The antioxidant levels are lower in RA patients [9]. An imbalance between Th1 and Th2 cells activity ratio increases Th17 cells activity and the level of cytokines mRNA secreted from Th0 or Th1 in peripheral blood; moreover, joint tissue is observed in patients who suffer from active rheumatoid arthritis [10]. The increase in IL2 and IL2/IL4 ratio are good indicators of increased Th1/Th2 ratio [8].

IL-6 has a pivotal role in the pathophysiology of RA [11]. This cytokine induces synovitis and joint destruction by provoking neutrophil migration, osteoclast maturation, and vascular endothelial growth factor-stimulated pannus proliferation [12].

Previous studies have shown that T cells play an important role in the development of such autoimmune disease. The balance between regulatory T cells and proinflammatory effector T cells has been shown to be of critical importance in the development and persistence of autoimmune diseases[6]. In R.A patients, elevated levels of the following cytokines and chemokines have been observed in many studies (interlukin-1, interlukin-2, interlukin-6. Though in many cases RA is a progressive joint disease, its course is variable and individual, and there is a lack of reliable tests to assess its outcome. Of the traditional markers of the disease process used in inflammatory arthritis, radiographs mostly measure irreversible changes, while the erythrocyte sedimentation rate (ESR) reflects inflammatory activity [13]. It can be postulated that a combination of biochemical risk factors and genetic polymorphism rather than a single test would have a better prognostic value for aggressive joint disease. Such an analysis could be used to differentiate patients who will need the most aggressive therapy from those who can be treated with DMARD monotherapy [14]. This project will done to evaluate the association of TGF-β1 polymorphism in the development and the severity of RA.

2. Materials and Methods

In this study the experimental group consisted of the of the Rheumatoid Arthritis patients. patients .This study was conducted at Ibn Al-Khatib hospital , Baghdad included 25(50%) male and 25(50%) female patients ,while the control group included 15(50%) male and 15(50%) female, range age between 10 to 60.

In this analytical meta-ct study, patients with Rheumatoid Arthritis at Ibn Zahra Hospital as well as Ibn Al-Khatib after confirming the injury, all WBC, ESR, Sugar, Urea, IL-1b, CD4, test levels were studied in basal blood sample employing impedance technology and photometry as measurement method using ABX micros 60 hemat analyzeologyr, Urea, Sugar semi-automated chemistry analyzer using Mindray BC-5000by linear kit. The data was analyzed with Graph pad prism software . .the measurement of the level of IL-2, of IL-6 by means of The enzyme-linked immunosorbent assay. Electrochemiluminescence Immunoassay (ELSIA) method using Shanghai Diagnostic HumaReader HS Autoanalyzer.

2.1 Statistical Evaluation

Information was investigated by utilizing Statistical Package for Social Sciences, Graphpad crystal Software. The outcomes were figured as mean ± standard deviation for quantitative factors utilizing t-test. On the whole factual investigation.

3. Results

The current study included 50 male and female patients suffering from rheumatoid arthritis. And a control group of 30 uninfected people, males and females. The study groups were distributed according to percentages for sex, age group, hematological and biochemical indicators, and how much they were distributed for the immune indicators.

Table (1) showed the mean age between control (healthy people) and patients with RA in the case of the total patients 50, the rate of the male was 25(45%) and women 25 (55%), while in the case of total control 30 non-individuals (controls group).

Table 1- characteristics of study groups

Age\Gender		**Patient**		**Control**	
		No.	**%**	**No.**	**%**
Age	10-60year	50	100 %	30	100 %
Gender	Male	25	45 %	15	50 %
	Female	25	55 %	15	50 %
	Total	50	100 %	30	100 %

P-value (PatientsVSControls) Pvalue<0.05

Table 2- Comparison of Hb , WBC and ESR levels in males and females in R.A. Patients from the studied population

Parameter	Sex	N	Mean	Std. Deviation	P value
Hb	Male	25	12.56	1.3	0.50
	Female	25	11.32	1.3	
WBC	Male	25	10.81	5.45	0.17
	Female	25	9.15	4.08	
ESR	Male	25	34.69	18.94	0.008**
	Female	25	50.13	25.54	

Table (2) showed higher hemoglobin levels in infected males (12.56±1.3) compared to females (11.32±1.3), with no significant difference. As for white blood cells, they increased in infected males (10.81±5.45) compared to infected females (9.15±4.08). With no statistical significance between genders ($p>0.05$). As for the ESR index, the current study showed higher levels in females (50.13±25.54) compared to infected males (34.69±18.94), with no statistically significant differences between the sexes ($p>0.05$).

Table 3- Comparison of Sugar, Urea, LDH and Creatinine levels in males and females in R.A patients of studied population.

Parameter	Sex	N	Mean	Std. Deviation	P value
Urea	Male	25	46.66	21.77	0.8
	Female	25	45.29	22.20	
Sugar	Male	25	159.50	83.12	0.55
	Female	25	148.00	70.07	
LDH	Male	25	330.95	15.85	0.001***
	Female	25	333.13	14.86	
Creatinine	Male	25	0.80	0,06	0.89
	Female	25	0.70	0,06	

Table (3) showed higher levels of urea, sugar, LDH and Creatinine in infected males (46.66±21.77), (159.50±83.12) , (330.95±15.85) and (0.80±0.06) compared to infected females (45.29±22.20) (148.00±70.07) , (333.13±14.86) and (0.70±0.06) . There was no significant difference in morality between genders (P>0.05).

Table 4- Comparison of IL-2, IL-6 levels in males and females in R. A. patients of studied population

Parameter	Sex	N	Mean	Std. Deviation	P value
IL-2	Male	25	982.37	244.87	0.93
	Female	25	974.44	480.96	
IL6	Male	25	3.42	1.33	0.22
	Female	25	3.88	1.69	

Table (4) showed higher levels of IL-2 in infected males (982.37±244.87) compared to infected females (974.44±480.96) .as well as showed higher levels of IL6 in infected females (3.88±1.69) compared to infected males (3.42±1.33) there was no significan moral difference in morality between sexes (p>0.05).

3. Discussion

The physiological study of rheumatoid arthritis indicates the interference of many molecules and cells Natural and acquired immunity in the emergence and progression of the disease and the immunopathological mechanisms of rheumatoid arthritis are not clear [15].

In the presence of unknown pathogens, a non-specific immune response occurs. The joint lesions in rheumatoid disease begin with an inflammatory lesion within the synovial membrane called pannus which is a proliferation of synovial tissue with infiltration of mononuclear cells, especially T lymphocytes [16]. This tissue invades the cartilage and bone surface of the joint, leading to the formation of erosions. Metalloproteases that degrade cartilage, humoral-mediated immune mechanisms with rheumatoid factors production of anti-IgG immunoglobulin's autoantibodies to a number of anti-nuclear, anti-cytoplasm, anti-collagen, antibodies, cellular-mediated with overactivity of helper lymphocytes in the synovial tissue membrane[16], in addition to the mechanisms by which various cytokines, in particular TNFα-1, and IL-6, are exerted by their specific effect.

Inflammatory IL-8 and its effect on multi nucleated immune cells as neutrophils. Inflammatory cytokines play a key pathogenic role in inflammatory processes, synovial tissue proliferation and cartilage degeneration. Within inflamed joints, an imbalance occurs between stimulating cytokines such as IL-6, IL-1, TNFα and anti-inflammatory cytokines represented by IL-11. This study supports what was indicated [17].

Although IL-2 does not directly mediate or aggravate the pain status of patients, in this study, RA patients' serum IL-2 levels were significantly positively correlated with their VAS

pain scores and levels of pain-related cytokines such as IL-6 and TNF-α. In this study, elevated serum IL-2 levels in RA patients paralleled their levels of pain[18].

References

[1] J. Smolen, D. Aletaha, A. Barton, G. Burmester, P. Emery, and G. Firestein, "Rheumatoid arthritis Nat Rev Dis Primers. 2018; 4: 18001," ed: Article.

[2] Z. Szekanecz, A. Koch, and P. Tak, "Chemokine and chemokine receptor blockade in arthritis, a prototype of immune-mediated inflammatory diseases," *Neth J Med*, vol. 69, no. 9, pp. 356-366, 2011.

[3] G. S. Firestein, "Evolving concepts of rheumatoid arthritis," *Nature*, vol. 423, no. 6937, pp. 356-361, 2003.

[4] S. T. Ngo, F. J. Steyn, and P. A. McCombe" ,Gender differences in autoimmune disease," *Frontiers in neuroendocrinology*, vol. 35, no. 3, pp. 347-369, 2014.

[5] H.-Y. Yap, S. Z.-Y. Tee, M. M.-T. Wong, S.-K. Chow, S.-C. Peh, and S.-Y. Teow, "Pathogenic role of immune cells in rheumatoid arthritis: implications in clinical treatment and biomarker development," *Cells*, vol. 7, no. 10, p. 161, 2018.

[6] S. B. Cohen *et al.*, "A multicentre, double blind, randomised, placebo controlled trial of anakinra (Kineret), a recombinant interleukin 1 receptor antagonist, in patients with rheumatoid arthritis treated with background methotrexate," *Annals of the rheumatic diseases*, vol. 63, no. 9, pp. 1062-1068, 2004.

[7] E. H. Choy, F. De Benedetti, T. Takeuchi, M. Hashizume, M. R. John, and T. Kishimoto, "Translating IL-6 biology into effective treatments," *Nature Reviews Rheumatology*, vol. 16, no. 6, pp. 335-345, 2020.

[8] N. Aryaeian, M. Djalali, F. Shahram, A. Djazayery, and M. R. Eshragian, "Effect of conjugated linoleic Acid, vitamin E, alone or combined on immunity and inflammatory parameters in adults with active rheumatoid arthritis: a randomized controlled trial," *International journal of preventive medicine*, vol. 5, no. 12, p. 1567, 2014.

[9] F. Javadi *et al.*, "The effect of quercetin on plasma oxidative status, C-reactive protein and blood pressure in women with rheumatoid arthritis," *International journal of preventive medicine*, vol. 5, no. 3, p. 293, 2014.

[10] M. Feldmann and S. R. N. Maini, "Role of cytokines in rheumatoid arthritis: an education in pathophysiology and therapeutics," *Immunological reviews*, vol. 223, no. 1, pp. 7-19, 2008.

[11] E. G. Favalli, "Understanding the role of interleukin-6 (IL-6) in the joint and beyond: a comprehensive review of IL-6 inhibition for the management of rheumatoid arthritis," *Rheumatology and therapy*, vol. 7, no. 3, pp. 473-516, 2020.

[12] S. Srirangan and E. H. Choy, "The role of interleukin 6 in the pathophysiology of rheumatoid arthritis," *Therapeutic advances in musculoskeletal disease*, vol. 2, no. 5, pp. 2.2010 ,256-47

[13] N. Peel, R. Eastell, and G. Russell, "Markers of bone and collagen breakdown in early inflammatory arthritis," *Baillière's clinical rheumatology*, vol. 6, no. 2, pp. 351-372, 1992.

[14] P. Nemec, M. Pavkova-Goldbergova, M. Stouracova, A .Vasku, M. Soucek, and J. Gatterova, "Polymorphism in the tumor necrosis factor-α gene promoter is associated with severity of rheumatoid arthritis in the Czech population," *Clinical rheumatology*, vol. 27, pp. 59-65, 2008.

[15] S. E. Gabriel *et al.*, "Survival in rheumatoid arthritis: a population-based analysis of trends over 40 years," *Arthritis & Rheumatism*, vol. 48, no. 1, pp. 54-58, 2003.

[16] A. Young and G. Koduri, "Extra-articular manifestations and complications of rheumatoid arthritis," *Best practice & research Clinical rheumatology,* vol. 21, no. 5, pp. 907-927, 2007.

[17] A. Bharadwaj and N. Haroon, "Interstitial lung disease and neuropathy as predominant extra-articular manifestations in patients with rheumatoid arthritis: a prospective study ",*Medical science monitor: international medical journal of experimental and clinical research,* vol. 11, no. 10, pp. CR498-502, 2005.

[18] A. Ateş, G. Kinikli, M. Turgay, G. Akay, and G. Tokgöz, "Effects of rheumatoid factor isotypes on disease activity and severity in patients with rheumatoid arthritis: a comparative study," *Clinical rheumatology,* vol. 26, pp. 538-545, 2007.