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ORIGINAL ARTICLE

Evaluation of Drug Effects on IL-17 and TNF levels in Patients with Rheumatoid Arthritis

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ABSTRACT

Background: *Rheumatoid arthritis (RA) is a chronic, autoimmune inflammatory illness considered a progressive joint injury and difficult to treat due to persistent synovitis. Patients suffer from swelling, and stiffness in many joints bilaterally.* ***Objective:*** *The purpose of this investigation is to the impact of medication on the levels of TNF-α and IL-17 in rheumatoid arthritis (RA) patients and to detect the differences in IL-17 and TNF-α levels in treated and untreated cases to determine the medication may affect these inflammatory markers in RA patients.* ***Methodology:*** *Seventy samples were collected from patients divided into (35 receive medicine and 35 without medicine) in the teaching Hospital of Alsadair. Thirty samples were collected from healthy people as control. The period of study between September 2023 to February 2024. RA was detected by clinical examination and laboratory investigations including anti-ccp, RF, serum interleukin-17 and TNF-α level.* ***Results****: This research revealed 20 (29.4%) women and 5 (54.5%) men. This study illustrated that patients without medicine gave a positive result for RF, while patients receive medicine had negative results compared with control had negative result ($p < 0.01$). Also the study showed that positive results for Anticcp in patients without medicine, while patients taking medicine were 31(88.5%) who showed a negative result for anticcp compare with control 30 (100%) ($p < 0.01$). Low levels of IL-17 and TNF (3.7±0.7and 2.4. ±0.04) respectively were detected in patients taking medicine compared with controls, while the elevated IL-17 levels and TNF appeared in patients without medicine (IL-17, 5.6± 0.23 and TNF 4.1±0.01) respectively (p-value < 0.05) compared with control. High amounts of IL-17 and TNF in patients with chemical treatment were (4.3±0.9 and 4.1±0.02) in contrast to the control respectively, also biological treatment indicates no significant difference in levels of IL-17 and TNF (3.1±0.01 and 2.2±0.01respectively). In addition, patients taking combination therapy did not show a noticeable change in IL-17(3.3±0.1) and TNF (2.1±0.01).* ***Conclusion:*** *RA affects women more than men, IL-17, and TNF-α can be used as diagnostic biomarkers and Anti-CCP is a better diagnostic guide due to their higher sensitivity and specificity for RA.*

INTRODUCTION

Small joints could be affected by the systemic autoimmune disease known as rheumatoid arthritis (RA). It is first thought to be inflammation and the formation of new cells in the synovial lining of the joints, which ultimately results in the degeneration of bone and cartilage[1].

Its symptoms are present in 20–50 cases per 100,000 adults annually, affecting 0.5–1% of adults worldwide. Women over forty are mostly affected, with the condition affecting women three times more frequently than men[2].

Smoking oxidative stress has been demonstrated to contribute to the initiation and perpetuation of local and systemic inflammation, which are important features of rheumatoid arthritis (RA). Although the exact cause of RA is still unknown, it is thought to be most likely caused by a combination of genetic predisposition and ecological triggers[3].

Also autoreactive T cells have a vital role in this illness, such as Th1 and Th17, B cells, macrophages, plasma cells alongside with cytokines (IL)-1, IL-2, IL-6, IL-17, interferon-gamma (INF-γ), and tumor necrosis factor-alpha ,(TNF-α) are pro-inflammatory cytokines that are stated more regularly in RA illness [4].

Fibroblasts, growth factors, chemokines, and adhesion - Th17 cells characteristically produce cytokines that play a crucial role in rheumatoid arthritis, such as IL-17 as well as IL-23, which are considered pro-inflammatory cytokines and support Th17 cell stimulation and diversity to generate IL-17[5].

There is only one cytokine family, which includes IL-17, along with IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25), and IL-17F[6].

IL-17 is crucial in promoting granulocyte production and mobilization. Granules facilitate movement via CXC chemokines, extending their survival in target tissues. Additionally, it causes certain target cells to produce nitric oxide (NO), IL-6, and cyclooxygenase-2 (COX-2)[7].

Though it has recently been identified as a factor that causes autoimmune diseases, TNF-α is thought to be a factor that causes necrosis. (TNF-α) attaches itself to two different types of receptors, which triggers the start of signal transduction pathways that result in cellular responses like cell survival, differentiation, and proliferation. Nevertheless, chronic inflammation and the advancement of pathological complications like autoimmune diseases are caused by the inappropriate stimulation of this (TNF-α)[8].

TNF-α that is hidden from Th1 cells and macrophages activates synovial fibroblasts, aids in epidermal hyperplasia, and attracts inflammatory cells. Following this, activation by different cytokines, such as TNF-α, IL-6, and IL-1 [9].

Collagen and proteoglycans are broken down by cathepsins and matrix metalloproteinases (MMPs), which are produced in greater quantities than normal by synovial fibroblasts. Because bone cells activate TNF-α, they also contribute to the development of rheumatoid arthritis diseases. Activated osteoblasts in rheumatoid arthritis also stimulate synovial hyperplasia and angiogenesis. As a result of the above, damage to cartilage and bones occurs, followed by joint erosion[10].

The lack of a viable treatment for rheumatoid arthritis makes it a challenging condition to treat. It is treated with a variety of hand-me-down medications, including glucocorticoids, nonsteroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs), and vulnerable natural mediators. Since they have anti-inflammatory, analgesic, and antipyretic properties, nonsteroidal anti-inflammatory drugs (NSAIDs) are the first line of treatment for rheumatoid arthritis. It is not, however, utilized as the main treatment for this illness because it does not [11].

Third-line treatment for rheumatoid arthritis involves the use of glucocorticoids, like betamethasone, which are potent anti-inflammatory drugs. They stop arthritis from progressing and promptly and efficiently lessen early symptoms[12].

Despite being used extensively to lower the incidence of rheumatoid arthritis, disease-modifying antirheumatic drugs (DMARDs) have a low efficacy rate and can take up to five months to show results. To postpone the onset of disease, they lessen synovitis and enhance cartilage repair[13].

The aim of the present work is to assess the impact of the medication on the levels of TNF-α and interleukin 17 (IL-17) in rheumatoid arthritis (RA) patients and to detect the differences in IL-17 and TNF-α levels in treated and untreated cases to determine whether the medication may affect these inflammatory markers in rheumatoid arthritis patients.

METHODOLOGY

Sample collection

In this study 70 samples were collected from individuals with illnesses rheumatoid arthritis divided into (35 that didn't receive medicine and 35 that received medicine) which were collected from the Teaching Hospital of Alasdair (Consultant of arthritis) as well as 30 samples were collected from healthy people as control the period of study between September 2023 to February 2024, patients by additional inflammatory autoimmune disease were excluded from the reading.

Immunological Study

RA was detected by clinical examination and studies conducted in laboratories counting anticcp was measured via enzyme-linked immunosorbent assay(ELISA) kit from Genius / USA protocol., and RF by latex method. Measurement of serum IL-17 level and TNF-α was achieved by a commercial ELISA KIT, ELISA method (sandwich enzyme-linked immunosorbent Assay) using ELISA technique reader (Human Reader HS. USA). The company of the kit is Solarbio, China. Following a fast of five milliliters, the cases' venous blood was extracted and allowed to agglutinate for approximately twenty minutes at room temperature. Anti-CCP antibodies were measured using an ELISA kit, and the blood was used to detect RF by RF latex slide agglutination (Solarbio/china). The serum was started by centrifugation at 3000 rpm for 10 minutes, and it was then stored at -20.

Statistical Analysis:

The Chi-square test and the arithmetic mean were used to calculate the variables. For the RF and Anti-CCP, a probability value of $P < 0.01$ was deemed statistically significant. On the IL-17 and TNF, a one-way ANOVA analysis was also performed, and the findings showed statistically significant differences (p-value < 0.05). Patients before and after treatment, as well as between patients before treatment and the control group, were found to differ from one another according to Tukey-HSD analysis.

RESULTS

Seventy blood samples were obtained from patients suffering from RA 35(50%) samples from patients without taking medicine, 35(50%) from patients taking medicine and 30 (100%) samples from healthy people as control. The gender represented 60 (85.7 %) females and 10 (14.3%) males (figure 1).

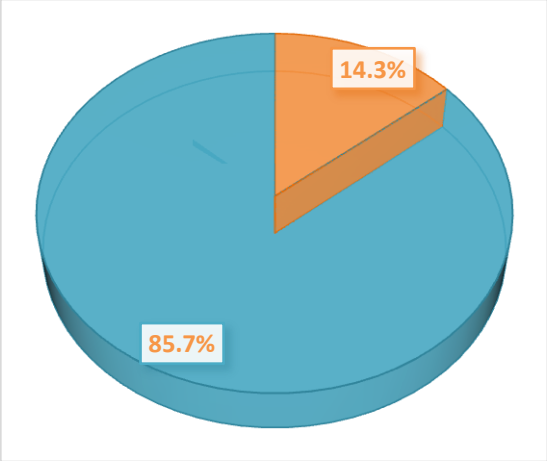

Fig.1: distribution of patients' gender for rheumatoid arthritis

This study showed the RA is widespread in most ages, but in varying proportions, with the highest percentage recorded in the age group (45-54) years, 20 (29.4%) women and 5(54.5%) men, followed by age group (35-44) years, 12(20.5%) women and 1(9.5%) men, while The percentage was lowest in the age group <25 for women 5(6.4%) and men 1(4.5%), the age group ≥65 appear 6(10.2%)females and 1(14.3%) males. There was a diversity in the rest of the age groups, as showed in Table 1.

Table 1: distribution of patients of RA according to the age

Age	Gender (%)		Total
	Male	Female	
<25	1 (1.4%)	5(7.14%)	6 (8.54%)
25-34	0(0%)	12(17.15%)	12 (17.15%)
35-44	1(1.4%)	12(17.15%)	13 (18.19%)
45-54	5(7.15%)	15(21.45%)	20(28.6%)
55-64	2(2.86%)	10(14.29%)	12(17.105%)
≥65	1(1.٤%)	6(8.52%)	7(11.92%)
Total	10(14.3%)	60(85.7%)	70(100%)

RF and ANTICCP in RA

Patients who were not given any medication included 30 out of 35 individuals (85.7%)tested positive for RF, while 10 individuals 5(14.3%) tested negative. In contrast, 30 out of 35 individuals (85.7%) tested negative for RF among patients taking medication, and 5 individuals (14.3%) gave a positive result. Thirty individuals in the control group had all of their RF results come out negative. The statistical analysis ($p < 0.01$) suggests a significant difference between the patient groups and the control group concerning RF. Anti-CCP All 35 individuals (100%) were positive for anti-CCP in patients who declined to receive any medication.

Conversely, among patients on medication, anti-CCP was not detected in 31 out of 35 (88.5%) cases. Anti-CCP results for the thirty members of the control group were all 100% negative. A significant difference in Anti-CCP between the patient groups and the control group is shown in table 2.

Table 2: percentage of RF and Anti-CCP in patients before and after treatment compared with control groups

Parameters	Before taking medicine No(%)		After taking medicine No(%)		Control No(%)		total	Chi-square	P value
	+	-	+	-	+	-			
RF	30(85.7%)	5(14.3%)	5(14.3%)	30(85.7%)	-	30(100%)	100	55.2	$p < 0.01$.
TOTAL	35(100%)		35(100%)		30 (100%)				
	100(100%)								
Anti ccp	35(100%)	-	4(21.5%)	31(88.5%)	-	30(100%)		85.1	$p < 0.01$.
TOTAL	35(100%)		35(100%)		30 (100%)		10		

The investigation's findings indicate that a high percentage of patients 15(45.7%) received chemical treatment followed by 12(31.4%) patients who received biology treatment and the less treatment was a combination of biology and chemical 8(22.8%) Figure 2.

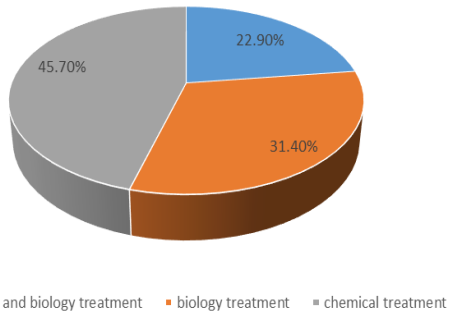

Fig .2: Shows the distribution of patients according to treatment

Significant differences were found between the levels of IL-17 and TNF in patients who were taking medication and those who were not. The results showed that patients who were taking medication had higher levels of IL-17 and TNF (5.6±0.23 and 4.1±0.01), respectively, compared to the control group, while patients who were taking medication had lower levels of both (3.7±0.7 and 2.4±0.04), respectively, compared to the control group (IL-17, 5.6±0.23 and TNF, 4.1±0.01) with (p-value < 0.05). Table (3).

Table 3: levels of IL-17 and TNF in RA patients compared with control

Parameter	Without Medicine Mean±SD	With Medicine Mean±SD	Control Mean±SD	P. value
IL-17	5.6± 0.23	3.7±0.7	3.1±0.02	p-value < 0.05*
TNF	4.1±0.01	2.4. ±0.04	2.2± 0.05	p-value < 0.05*

* = significant

For the amounts of IL-17 and TNF in patients taking chemical treatment their result was 4.3±0.9 and 4.1±0.02 in contrast to the control respectively, which indicates that there was a significant contrast between the control and patients taking chemical treatment, which shows that chemical treatment significantly didn't affect these cytokines' levels. This indicates that chemical treatment may not be sufficient to modify the inflammatory cytokines IL-17 and TNF, also biology treatment shows no significant difference in levels of IL-17 and TNF (3.1±0.01 and 2.2±0.01respectively) between the control and patients taking this treatment, the biological treatment significantly affects these cytokines' concentrations. So This indicates that biological treatment may be sufficient to modify the inflammatory cytokines IL-17 and TNF. In addition, patients taking combination therapy did not show a discernible change in the degree of IL-17(3.3±0.1) and TNF (2.1±0.01) in contrast to the control group.

Our result indicates that the combination of chemical treatment and biological treatments significantly affects these cytokines' levels, also, the significant p-value indicates that the synergistic effect of combining chemotherapies and biological treatments is effective in controlling the inflammatory response, as evidenced by variations in the levels of TNF and IL-17and the combination could be more effective in managing inflammatory conditions characterized by elevated levels of IL-17, and TNF as shown in Table 4.

Table 4: Correlation between IL-17, and TNF and treatment

Parameter	Treatment(Mean ± SD)				P
	Chemical	Biology	Combination	Control	P value
IL-17	4.3±0.9	3.1±0.01	3.3±0.1	3±0.03	P<0.05
TNF	4.1±0.02	2.2±0.01	2.1±0.01	1.9±0.05	P<0.05

DISCUSSION

In this work, our results coordinate with Zhao et al [12] who found that RA ratio was more in female than male of 3:1 ratio. The RA sufferers displayed a high increase in standards for RF, and ACCP in contrast to the control group [14]. Also, a whole of 83 (52.2%) and 99 (62.3%) patients had anti-CCP antibody and RF, separately [15]. IL-17 level was greater in the RA group (226.6±215.6 pg/ml) than the control group (48.17±54.9pg/ml) with p-value of <0.001[16]. Hashiam and Aldahhan[1] showed that RA patients had higher mean serum levels of IL-6 and TNF-α (197 & 308), which were individually linked to the control group (5 & 57 pg/ml) with a significance level of p ≤0.05.

Shin et al [17] characterized rheumatoid arthritis as being two to three times more common in women than in men, due to numerous reasons, including that autoimmunity is subject to genetic control, as these genes have a primary role in the illness's dissemination among women. Sex hormones, however, play a significant part. The immune response may be controlled in this disease, particularly in relation to estrogen, by encouraging the survival of self-reactive clones that are prohibited, which would ultimately cause autoimmunity in women[18]. Rheumatoid arthritis is caused by antibodies against citrullinated proteins, which are targeted by the immune system in certain body tissues. Citrulline plays a significant role in numerous infections, which in turn trigger an immune response against these proteins [19]. According to studies, white blood cells are crucial for the onset and progression of rheumatoid arthritis. Synovial tissue is the primary site of cytokine production by monocytes and macrophages, which is how inflammation is initiated[20] .

Interferon-γ, IL-2, and TNFα are among the inflammatory particles released by Th1 that stop CD4+ T cells from differentiating into Th2 cells. Apart from being linked to rheumatoid arthritis, Th17 is primarily responsible for producing cytokines such as TNFα, IL-6, and IL-17A, which are elevated in the serum of patients with rheumatoid arthritis and stimulate other cells [21].

TNF-α stimulates synovial fibroblasts, which results in an overabundance of cathepsin production. This, in turn, causes collagen and proteoglycans to break down, destroying cartilage and bones and eroding joints. In rheumatoid arthritis, osteoblasts promote angiogenesis and synovial hyperplasia. TNF-α is secreted by macrophages and activated dendritic cells (DCs). Additionally, TNF-α stimulates osteocytes, which results in the production of synovial fibroblasts and joint erosion[22]. Synoviocyte formation and the synthesis of cytokines and chemokines, particularly IL-6 and IL-8, are stimulated by IL-17A. Furthermore, IL-17 promotes the migration of synovial cells and facilitates the invasive phenotype that causes tissue damage. Bone erosion and the breakdown of cartilage matrix are examples of tissue damage. The matrix is practically destroyed by MMP. MMP-1, −2, −9, and −13 caused RA synovial cells and chondrocytes to release IL-17. Osteopathy. In the end, RANK and osteoclastogenesis activation cause osteoclasts to erode bone[23].

Disease-modifying antirheumatic drugs (DMARDs) are pharmaceuticals that are synthetic or chemical-based and can also be used to treat rheumatoid arthritis. By weakening the immune system and lowering inflammation, these drugs relieve RA symptoms and delay its progression[24], however, the lack of success in stopping the progression of the disease means that the response to this treatment is inadequate[25]. Biological medications specifically target immune system components involved in the inflammatory response. Patients who have not responded well to multiple DMARD regimens or who have experienced insufficient response to treatment have been referred to use biological drugs. TNF blockers (adalimumab, etanercept, infliximab, and golimumab) are among the biological therapies used.Rituximab, 4-mediated costimulation (abatacept), and cytotoxic T lymphocyte antigen (CTLA) are three examples of this. Moreover, when receiving biologic therapy in addition to related DMARD treatment, a TNF blocker is added. However, it has been discovered that 30–40% of patients do not respond to TNF blockers, and the few who do generally experience a total reduction in their reactions, with many eventually losing them[26].

CONCLUSION

TNF and IL-17A are the causes of the persistent localized synovial inflammation, particularly in rheumatoid arthritis. Both the early and late phases of rheumatoid arthritis are influenced by TNF and IL-17A. When treating inflammation, it's critical to inhibit TNF and IL-17 signaling. Since rheumatoid arthritis (RA) is treated with immunosuppressants and addresses both clinical symptoms and organic manifestations, RA contributes to the improvement of this disease.

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Declaration

Author's contribution

Conceptualization: Abed Alhamza, K. R., & Ruaa, S. H. Data curation, Formal analysis, Investigation, Project administration, Validation, Visualization, Writing-original draft: Abed Alhamza, K. R., Methodology: Abed Alhamza, K. R., & Ruaa, S. H. Software: Abed Alhamza, K. R., & Ruaa, S. H.. Supervision: Ruaa, S. H.. Writing- review & editing: Abed Alhamza, K. R., & Ruaa, S. H. authors have reviewed and approved the final version of this article before publication.

Ethical approvals

This project has been reviewed and approved by the scientific Committee of the Department of Pathological analyses/ Faculty of Science/University of Kufa, and the laboratory Department of the Iraqi healthy ministry on August 23,2023. Written informed consent was obtained from each participation in the current study.

Conflict of Interest:

The authors declare that they have no financial support. The authors declare no conflicts of interest for research, authorship and publication of this article.

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