The Inflammatory Cytokines in Rheumatoid Arthritis and Their effect in the Progression and Pathogenesis of the Disease

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KEYWORDS
CRP, TNF-α, Inflammatory Cytokines, IL-6, IL-1β, Rheumatoid Arthritis

ABSTRACT
Background: Rheumatoid arthritis (RA) is a systemic autoimmune disorder affected the entire body.
Aims of the study: Estimation of levels of inflammatory biomarkers in patients with RA and investigation of their impact on the development and severity of the disease.
Methodology: This trial is a case-control one involving 44 patients with RA and 44 healthy age matched controls. Blood samples (5 ml) were collected from each participant, anti-CCP, RF, CRP, and ANA levels were measured using a Cobas device, while TNF-α, IL-10, IL-6 and IL-1β, were determined by an ELISA assay.
Result: The results showed no significant variations in age, place of residence, and smoking between the RA and control groups, while there were significant variations in BMI, gender, and professional status. The levels of ESR, anti-CCP, RF, ANA, and CRP were significantly elevated in the RA group ($P<0.001$ for each) than in the control group. The study of cytokine levels also showed significant variations between the two groups, with an increase in TNF-α, IL-6 and IL-1β, and a decrease in IL-10 in the patients. IL-1β was positively correlated with age, IL-6, IL-10, and TNF-α.
Conclusions: The results indicate that cytokines levels such as TNF-α, IL-6, IL-1β, and CRP are elevated in RA patients, reflecting elevated inflammatory activity, while levels of IL-10 are decreased, which may contribute to the exacerbation of the disease.

1. Introduction

RA, is an autoimmune disorder affected the entire body. The synovial joints are the principal targets of this chronic inflammatory condition, which is often the outcome of the complex interplay among several environmental and genetics variables (3). It begins in small, symmetrical joints on the periphery and, if untreated, it will spread to the proximal joints. Deterioration of the joint caused by chronic inflammation eventually results in bone erosions and cartilage loss. RA worsens with time and increases the risk of death if left untreated 1-2. Rheumatoid arthritis (RA) affects around 0.24% of the population all over the world. The prevalence of RA is higher in areas with a large European population, including North America, Western and Northern Europe, and Australia. East Asia and Africa have far lower prevalence rates compared to Central and South America 3-4.

Tumor necrosis factor (TNF-α) influences many cell types in unique ways and possessed an essential effect in the onset of inflammatory disorders such as RA. There is mounting evidence that both trans-membrane and soluble forms of TNF-α contribute to the inflammatory response. Unlike soluble TNF-α, which operates independently of the cells that produce it, trans-membrane TNF-α functions through direct cell-to-cell contact. Mice that were engineered to produce trans-membrane TNF-α exhibited inflammation and synovial hyperplasia, and arthritis symptoms 5-6. Macrophages and T-helper 1 cells secrete TNF-α, which attracts inflammatory cells, thickens the epidermis, and promotes synovial fibroblasts 7. Activation of synovial fibroblasts by cytokines including IL-1, IL-6 and TNF-α cause an overexpression of cathepsins and matrix metalloproteinases (MMPs), which in turn degrade collagen and proteoglycan. Erosion of the joints occurs as a result of the cartilage and bone breakdown. In rheumatoid arthritis (RA), osteoclasts are vital because they activate TNF-α, which promotes angiogenesis and synovial hyperplasia 8. However, synthetic 9, and natural 10-12, anti-cytokines showed beneficial effects in the treatment of RA. The current research aims to estimate the inflammatory biomarkers levels in patients with RA and investigate their impact on the development and severity of the disease.

2. Methodology

This trial is a case-control trial carried out on (44) patients with RA and (44) age-matched healthy persons as control group. All patients was diagnosed by specialist physician during their visit to Nassiriya Teaching hospital through the period from November 2023 to March 2024. Verbal consent was taken from participants for blood collection and information recording. 5 ml blood sample was taken from all participant. Sera were isolated in special laboratory tubes and kept at -20 °C until use. 2 ml of blood was placed in an EDTA tube for measuring ESR levels. Anti-CCP (U/mL), CRP (mg/L), RF (IU/mL), and ANA levels were determined using a Cobas device according to the recommendations of (Roche, Germany). TNF-α,, IL-10, IL-6 and IL-1β levels were calculated using ELISA test (Sunlog, China).

Statistical analysis

The statistical analysis was carried out by using SPSS (version 26) and using dependent t-tests (two-tailed) and independent t-tests (two-tailed) for normally distributed variables, whereas the Mann-Whitney and Wilcoxontests were used for those variables that were not normally distribute. P value < 0.05 in any parameter was considered significant.

Ethical approval:

All participants who are going to be part of this study were properly informed and gave their verbal permission. This research was registered in, and approved by the postgraduate studies of Southern Technical University- Basrah. The committee on publication ethics at the Al-Naseriah teaching hospital, Thi-Qar, Iraq,also gave its approval to carry out the study.

3. Results and discussion

Socio-demographic and anthropometric characteristics

Table (1) regarding socio-demographic characteristics in the three groups (RA, and control), it show a non- significant difference in each of age, residence and smoking among the three groups. While there is a significant difference regarding BMI, sex and employment status.

Table 1: Demographic proprieties of RA patient and healthy control

Variables		Control group n=44	Rheumatoid Arthritis n=44	P. value*
Age (years)		47.41 ± 14.51	44.82 ± 12.14	NS*
BMI(kg/m^2)		26.98 ± 4.11	28.18 ± 4.53	NS*
Sex	Male	24	12	<0.01**
	Female	20	32	
Residence	City	36	34	NS**
	Village	8	10	
Smoking	Smoker	7	4	NS**
	Non-smoker	40	40	
Employment Status	Employee	27	11	<0.05**
	Private Job	0	1	
	Housewife	12	27	
	Not working	5	5	

** Chi-square test NS: Non-Significant

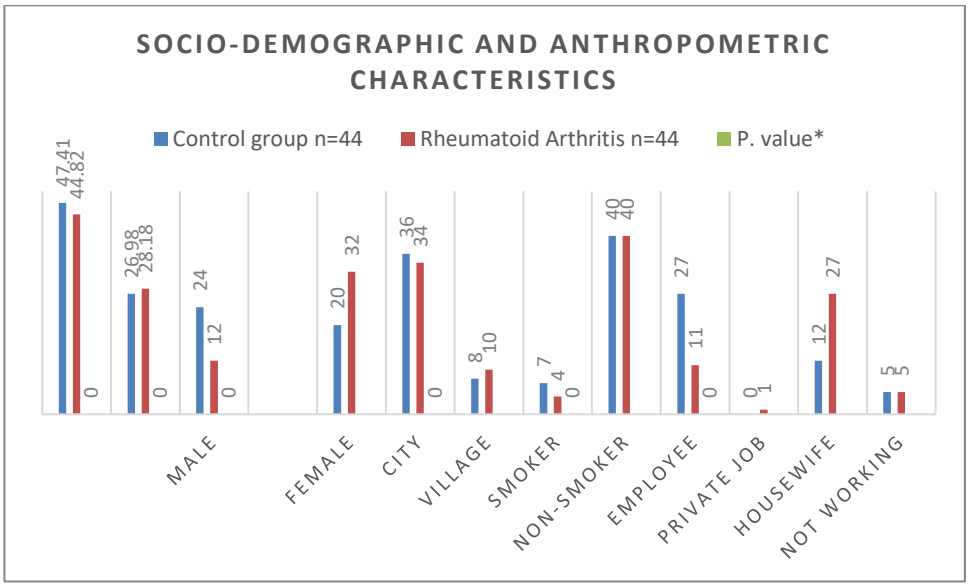

Figure 1: Demographic proprieties of the studied groups

Comparison of Inflammatory Markers Between RA Patients and Control

The results of Table 1 showed significant variations between the rheumatoid arthritis group and the control group in all measured inflammatory parameters. The mean erythrocyte sedimentation rate (ESR) in the rheumatic group was significantly higher (45.7 ± 20.3 mm/hr) than in the control (10.2 ± 5.1 mm/hr) with a P value less than 0.001. The levels of anti-CCP (90.4 ± 35.2 U/mL) and RF (80.3 ± 30.1 IU/mL) in the rheumatoid arthriis group were higher significantly than in the control group (4.8 ± 2.5 U/mL and 8.9 ± 4.3 IU/mL, respectively) with a P value less than 0.001 for each. In addition, ANA levels (70 ± 15%) in the rheumatic group showed a significant increase compared to the control group (10 ± 5%) with a P value less than 0.001. Finally, CRP levels (25.6 ± 12.4 mg/L) in the rheumatic group were significantly higher compared to the control group (3.1 ± 1.5 mg/L) with a P value less than 0.001, indicating a higher inflammatory activity in rheumatic patients.

Table 2: Mean ± SD and P Value for ESR, Anti-CCP, ANA, RF, and CRP Levels

Parameter	Rheumatoid Arthritis Group	Control Group	P Value
ESR (mm/hr)	45.7 ± 20.3	10.2 ± 5.1	<0.001
Anti-CCP (U/mL)	90.4 ± 35.2	4.8 ± 2.5	<0.001
ANA (titer)	1:160 ± 1:80	1:40 ± 1:10	<0.001
RF (IU/mL)	80.3 ± 30.1	8.9 ± 4.3	<0.001
CRP (mg/L)	25.6 ± 12.4	3.1 ± 1.5	<0.001

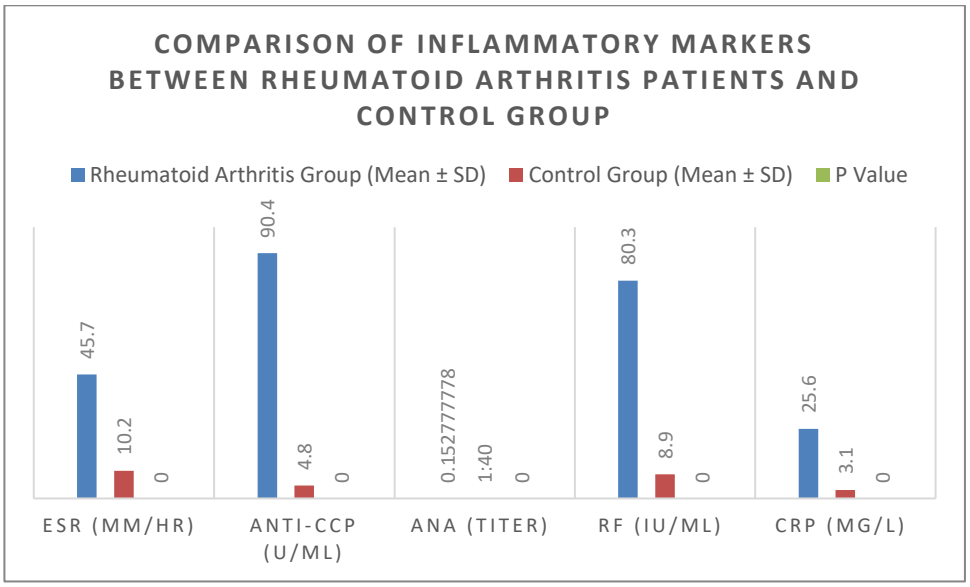

Figure 2: Comparison of Inflammatory Markers Between Study group

Comparison of cytokine levels between control groups and rheumatoid arthritis patients

The study that compared the average levels of some cytokines in the control group versus rheumatoid arthritis patients shows statistically significant differences. For IL-1β, its average levels in healthy subjects were 12.01 ± 2.56 versus 28.10 ± 8.44 in rheumatoid patients (P<0.001). For IL-6, readings were 4.10±1.64 versus 9.35±5.22 (P<0.001). For IL-10, there was a decrease in its levels among rheumatoid patients compared to the control group (13.62 ± 5.73 vs. 16.62 ± 4.22) with P = 0.01. Finally, TNF-α was at 16.41 ± 5.96 in the control group versus 22.67 ± 10.56 in the rheumatoid patients (P<0.01). These values indicate significantly elevated inflammation in RA patients compared to healthy people.

Table 3: The cytokine levels between control groups and rheumatoid arthritis patients.

Parameters	Control group (n=44)	RA group	P. value
IL-1β	12.01 ± 2.56	28.10 ± 8.44	<0.001
IL-6	4.10 ± 1.64	9.35 ± 5.22	<0.001
IL-10	16.62 ± 4.22	13.62 ± 5.73	0.01
TNF-α	16.41 ± 5.96	22.67 ± 10.56	<0.01

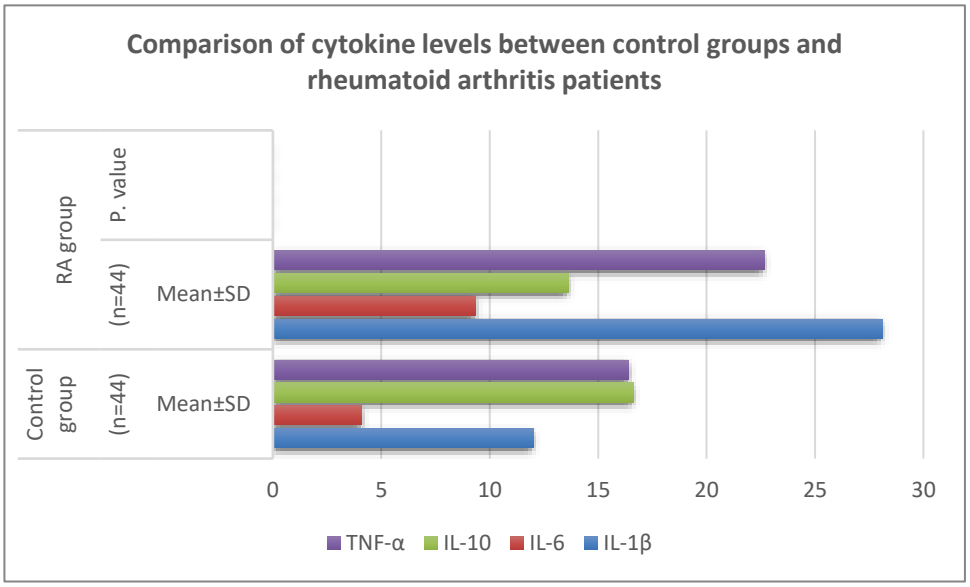

Figure 3: The cytokine levels between control groups and rheumatoid arthritis patients

Correlation of the study markers among rheumatoid arthritis patients

Table (3) regarding the correlation of the study markers among rheumatoid arthritis patients, it's show significant positive correlation (P<0.05) among IL-1β and each of age, IL-6, IL-10 and TNF-α (r=0.337, r=0.365, r= 0.485 and r=0.577) respectively. It is also showed that IL-6 and TNF-α were positively correlated (r= 0.503).

Table (4) Correlation of the study markers among rheumatoid arthritis patients

Parameters		Age	BMI	IL-6	IL-1β	IL-10
BMI	**Pearson Correlation**	-.006				
	Signif. (2-tailed)	.969				
IL-6	**Pearson Correlation**	-.031	.121			
	Signif. (2-tailed)	.849	.450			
IL-1β	**Pearson Correlation**	.337*	.043	.365*		
	Signif. (2-tailed)	.029	.785	.022		
IL-10	**Pearson Correlation**	.125	.070	.194	.485**	
	Signif. (2-tailed)	.460	.679	.271	.003	
TNF-α	**Pearson Correlation**	.220	.134	.503**	.577**	.237
	Signif. (2-tailed)	.234	.472	.005	.001	.234

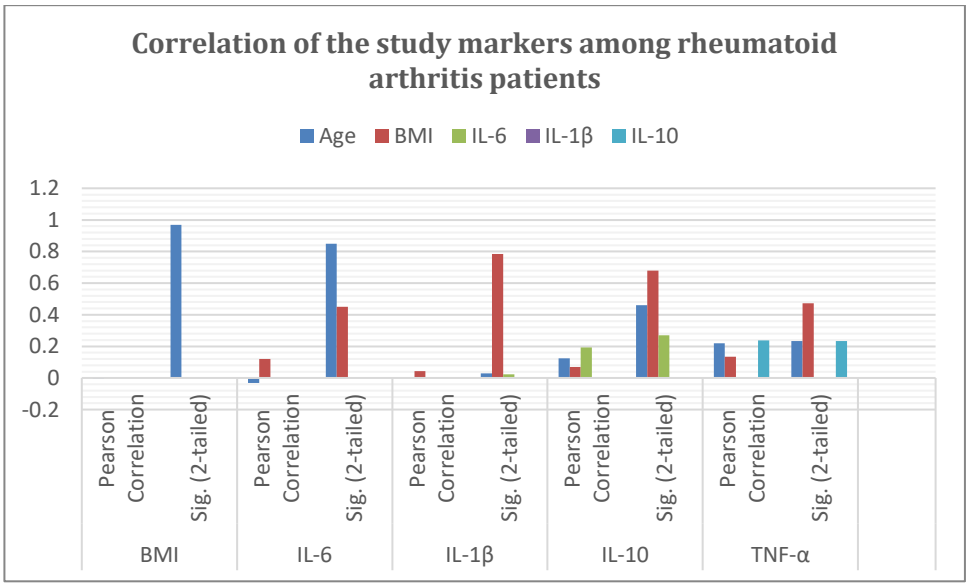

Figure 4: Correlation of the study markers among rheumatoid arthritis patients

Discussion

IL-1β and IL-1α are produced as pro-proteins that are tethered to the cytoplasm in the absence of signal peptides. Unlike pro–IL-1α, the biologically inactive pro–IL-1β requires caspase 1, for processing. The physiological impacts of IL-1β depend on its processing by caspase 1, which is linked to its release. In addition, IL-1 undergoes a similar process. The IL-1 biological action is dependent on a two-step process. These effects are activated by the 'inflammasome' complex: first, mRNA induction occurs, followed by the processing of the pro-molecule before it is secreted by the cells [13].

Our results revealed higher levels of IL-1β in individuals with RA compared to healthy control, and this is agree with Lee et al. (2021)[14], and Yang et al. (2019)[15].

Although the exact cause of rheumatoid arthritis is still uncertain, both clinical and experimental evidences suggested that IL-1β could play a key role in its development [16]. It has been noted that patients who have been diagnosed with rheumatoid arthritis display increased concentrations of the

cytokine IL-1 in their circulatory systems. Studies have shown that administering an IL-1 antagonist to these patients resulted in positive results. A prior study demonstrated that IL-1 receptor antagonist medication is not only safe and well-tolerated, but also has the ability to modify immune responses, resulting in clinical advantages [16].

IL-6 plays possesses a significant impact in the RA associated inflammatory events. Other cytokines (oncostatin M and adiponectin), belonging to the family of IL-6, are also participate in the inflammatory events [17].

The results also revealed a significant elevation in the IL-6 level in the RA patients. These results are consistent with Srirangan & Choy (2010) [18]. Rheumatoid arthritis is distinguished by a complex cytokines network, with an excess of cytokines found in both the bloodstream and synovial joints. One of these substances is IL-6, the essential mediator for the development of B-cells and the creation of auto-antibodies. It also directly triggers the release of CRP from liver cells, suggesting that it may exert a significant impact on the rheumatoid arthritis development. IL-6 plays also participates in the Th17 cells development in an animal models of autoimmune disorders. The various joint and whole-body symptoms observed in the confirmed RA patients can be attributed to the biological impact of IL-6[19-20].

Regulatory cytokines, such as IL-10, are produced by various immune cells and serve as anabolic stimulants. The receptor of interleukin 10 (IL-10 R1 and R2 subunits), is present on both immune and non-immune cells [21].

This study recorded a significant decrease in the IL-10 levels in RA patients compared to healthy controls. This finding is in agreement with that recorded by Li et al. (2016)[22]. IL-10 has been tested for its efficacy in mitigating inflammation and safeguarding cartilage in the management of RA, but,the outcomes were inconclusive. IL-10 exacerbates inflammation by enhancing the activity of B cells and the antigen-presenting cells contents of Fc receptors. This, in addition to the short $t_{1/2}$ of IL-10, has been cited as a potential explanation for the relatively underwhelming outcomes. IL-10 enhanced the Fc receptors expression on the bloodstream myeloid cells of rheumatoid arthritis patients who received IL-10 treatment [23]. IL-10 plays a vital role in protecting cartilage. It enhances collagen type II and aggrecan synthesis, which are crucial proteins in the cartilage extracellular matrix. Additionally, it alters the metabolic processes of proteoglycans, reduces the activity of MMPs, and inhibits the apoptosis of chondrocytes, similar to IL-4 [24].

Our findings showed a notable elevation in TNF-α levels in individuals with rheumatoid arthritis compared to the control group. This result in agreement with Moelants et al. (2013)[25] and Koyama et al. (2021) [26].

TNF-α is a primary cytokine in the RA progression due to its presence in considerable quantities in synovial tissue, while being absent in the synovial tissue in and in SLE. Moreover, there is a positive link between TNF-α level in the synovial tissues of individuals and the degree of bone degradation and inflammation in RA. The actions of TNF-α are attributed to its direct effect on several organs and its ability to enhance IL-8, IL-6, and IL-1 cytokines production [27-28]. Both TNF-α and IL-6 acted as pivotal mediaters in the progression of rheumatoid arthritis. Consequently, both anti-cytokine therapy and the cytokines themselves have been extensively studied in RA clinically and experimentally [29]

4. Conclusion and future scope

The results indicate that the cytokines such as TNF-α, IL-6, and IL-1β, as well as CRP were elevated significantly in RA patients compared to the healthy control, reflecting elevated inflammatory activity associated with disease exacerbation. In contrast, level of IL-10, was decreased significantly, which may contribute to disease severity. These results highlighted the importance of cytokines in the pathogenesis of RA and may help to guide treatment strategies.

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