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Comparative Analysis of TNF-A, IL-6 and IL-32 Levels between Rheumatoid Arthritis Patients and Controls by Age Group

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Abstract **Background:** Rheumatoid arthritis (RA) is an inflammatory illness characterised by chronic systemic inflammation that causes gradual joint destruction and can result in permanent disability. The pathogenesis of RA comprises a complicated network of different cytokines and cells that stimulate synovial cell growth and cause damage to both cartilage and bone. **Methods:** Study conducted between January and June 2025, in a private medical centre in Diyala/ Iraq. The study has been enrolled 89 participants, comprising 59 patients diagnosed with rheumatoid arthritis (29 males and 30 females) and 30 healthy controls (15 males and 15 females). TNF-a, IL-6 and IL-32 have been measured by using ELISA Quantitative kits. **Results:** The results showed elevated levels of the inflammatory cytokines TNF-α, IL-6 and IL-32 in rheumatoid arthritis patients compared to healthy individuals, although these differences did not reach statistical significance ($p>0.05$). Descriptively, elevated TNF-α and IL-6 levels were observed in participants aged 10–19 years and elevated IL-32 levels in participants over 60 years of age, with no statistically significant differences between the age groups ($p>0.05$). **Conclusion:** The study showed a tendency for higher levels of the inflammatory cytokines TNF-α, IL-6 and IL-32 in rheumatoid arthritis patients compared to healthy individuals, but these differences did not reach the level of statistical significance.

Key Words TNF-A, IL-6, IL-32, Rheumatoid Arthritis, Cytokines, Inflammatory Biomarkers, Autoimmune Diseases, Immunological Markers

INTRODUCTION

Rheumatoid Arthritis, considered an autoimmune inflammatory disease that affects multiple peripheral joints, may lead to chronic and persistent pain. It occurs due to the continuous inflammation of the synovial membrane. The main causes for this case are the elevation of anti-citrullinated protein antibodies (ACPAs) and rheumatoid factor (RF) production. In turn, this leads to deformities in multiple joints [1].

The global prevalence of rheumatoid arthritis is approximately 0.5–1% of the population for both types of infected mild, acute or severe arthritis. However, the arthritis patients, often suffering from joint destruction, which is causing severe pain in many cases, may lead to profound physical disability [2]. Furthermore, RA patients suffering from a vast range of physiological symptoms, involving weight loss, fatigue, loss of appetite, osteoporosis and muscle weakness [3]. Mortality rates among people with RA have been recorded from 0.45 to 0.46 per 100,000 of person [4].

Pro-inflammatory cytokines play a biological role in inducing the immune response to attack foreign bodies in the body. However, this role is not permanent; rather, it is organized by a accurate balance between pro- and anti-inflammatory cytokines, which preserve the stability of the immune system and prevent excessive or chronic responses [5]. If there is an imbalance between pro- and anti-inflammatory cytokines will occur, it may lead to damage the regulation of the immune response also participates in the development of chronic inflammatory diseases, like rheumatoid arthritis. This imbalance, in turn, may induce autoimmunity by enhancing the production of rheumatoid factors (RF) and anti-citrullinated peptide antibodies (ACPAs), under the impact of the main Pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) [6]. In rheumatoid arthritis, chronic inflammation of the synovial membrane caused by autoimmunity leads to increased formation and activity of osteoclasts, resulting in bone erosion and joint damage. Pro-inflammatory cytokines such as TNF-α, IL-6 and IL-32 are

also involved-α, released by activated macrophages and T-cells, exerts pro-inflammatory effects by binding to one of its receptors, p55 (TNF-a-RI) or p75 (TNF-a RII) and plays a critical role in producing other cytokines and inducing chronic inflammation [7,8].Another pro-inflammatory cytokine, IL-6, stimulates the development of antibodies and local synovial leukocytes' lymphocytes, natural killer cells, epithelial cells and blood monocytes all produce IL-32, a cytokine that was recently discovered. IFN-γ significantly increases IL-32 levels in epithelial cells and monocytes. Human recombinant IL-32 demonstrates various characteristics of proinflammatory cytokine [9]. Studies indicate that interleukin-32 (IL-32) not only acts as a pro-inflammatory cytokine but also contributes to amplifying the inflammatory response by stimulating the production of several other cytokines, such as TNF-α, IL-1β and IL-6, leading to persistent synovial inflammation and promoting cartilage and bone erosion. Furthermore, some studies have shown elevated IL-32 expression in the synovial tissues of rheumatoid arthritis patients, suggesting its potential use as a biomarker for disease activity and severity [10]. IL-32 can increase IL-1β and IL-6 production by 10 times when induced by muramyl dipeptides via NOD1 and NOD2, via a caspase-1-dependent mechanism [11]. A single NOD2 mutation affects a subset of Crohn's inflammatory bowel disease patients. Together, studies indicate that IL-32 plays a significant role in inflammation, both during host defence against pathogens and in autoimmune disorders [11,12]. This study aims to evaluate the levels of inflammatory cytokines (TNF-A-α, IL-6, IL-32) in the blood of rheumatoid arthritis patients and compare them according to age and gender groups. Where the study hypothesized that the levels of the inflammatory cytokines TNF-α, IL-6 and IL-32 are higher in patients with rheumatoid arthritis compared to healthy individuals and that these levels may vary according to age and gender.

METHODS

Study Design and Participants

This cross-sectional study was conducted at a private rheumatology clinic in Diyala Governorate, Iraq, from January to June 2025. Patients were recruited sequentially from the clinic's rheumatology outpatients throughout the study period, while the control group consisted of healthy individuals without inflammatory or autoimmune diseases. The study received ethical approval from the Research Ethics Committee at the College of Science, University of Diyala and all participants provided written informed consent before enrolment.

The study included 89 participants: 59 newly diagnosed rheumatoid arthritis patients and 30 healthy individuals as the control group. Patients were diagnosed by a rheumatologist based on clinical assessment and laboratory results, which included positive anti-CCP, RF, ESR, CRP and CBC.

The sample size was determined based on the number of patients who were eligible and willing to participate during the study period.

Inclusion and Exclusion Criteria

The study included patients newly diagnosed with rheumatoid arthritis by a rheumatologist who consented to participate. Individuals with other inflammatory or autoimmune diseases, urinary tract infections, chronic illnesses or seasonal influenza were excluded due to potential influences on the studied cytokine levels.

Statistical Analysis

The data were analysed using SPSS Statistics version 21. Descriptive data were presented as mean ± standard error for continuous variables, while categorical variables were presented as frequencies and percentages. The chi-square test was used to examine the relationship between categorical variables, such as the distribution of sex and age groups, between the patient and healthy groups. Statistical significance was defined as $p<0.05$.

RESULTS

The study found that both male and female patients were between 30 and 39 years old; 11 were female and 9 were male.

Table 1: Comparison between Patients with Arthritis study groups and control according to sex male By (Chi-square)

	Groups			
Age (years)	Patients' Male	Controls	Total	p value
10-19	4	4	8	0.19
20-29	6	3	9	
30-39	9	3	12	
40-49	7	2	9	
50-59	2	3	5	
≥ 60	1	0	1	

Table 2: Comparison between the Arthritis Patients study groups and the control according to sex female By (Chi-square)

	Groups			
Age (years)	Patients' Female	Controls	Total	p value
10-19	6	4	10	0.09
20-29	4	5	9	
30-39	11	2	13	
40-49	8	1	9	
50-59	0	3	3	
≥ 60	1	0	1	

Table 3: Distribution of TNF-A, IL-6 and IL-32 level Patients with age for male and female those affected.

Parameter	Age	Mean	Std. Error	p value
TNF-A	10-19	54.4	1.98	0.11
	20-29	53.6	1.17	
	30-39	34.67	3.89	
	40-49	32.09	1.45	
	50-59	26.27	10.34	
	≥ 60	22.16	11.76	
IL-6	10-19	42.13	1.65	0.13
	20-29	42.04	1.49	
	30-39	34.51	2.71	
	40-49	32.56	2.57	
	50-59	25.78	3.31	
	≥ 60	22.52	11.37	
IL-32	10-19	149.59	1.62	0.24
	20-29	148.56	1.32	
	30-39	147.67	2.68	
	40-49	145.89	2.68	
	50-59	133.45	3.91	
	≥ 60	232.45	22.70	

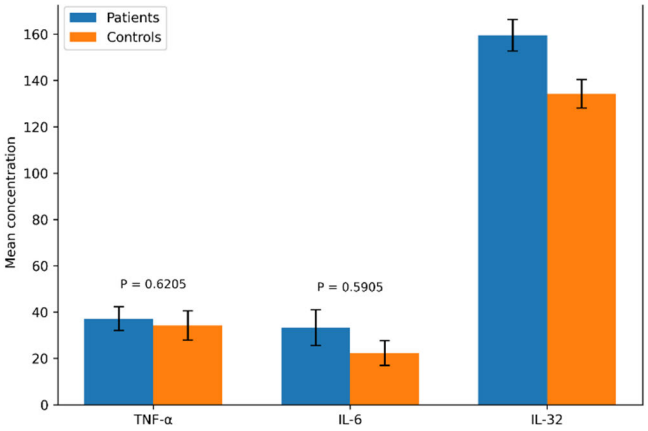

Figure 1: Comparison of mean TNF-α, IL-6 and IL-32 levels between rheumatoid arthritis patients and healthy controls.

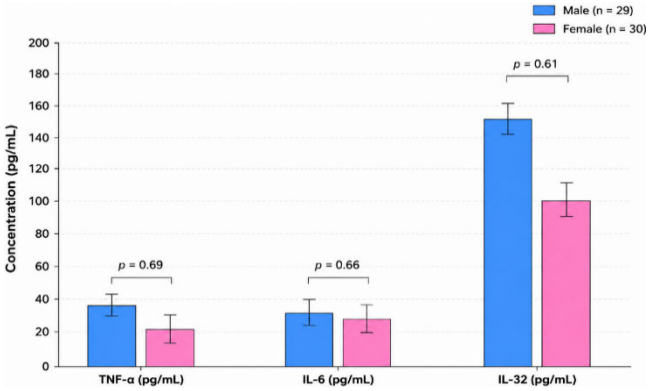

Figure2: Distribution of TNF-A, IL-6 and IL-32 level patients with gender for those affected

In comparison, the control group was 2 females and 3 males. There were no significant changes, as shown in Tables 1 and 2.

Regarding the distribution of TNF-A, IL-6 and IL-32 levels by age, the study found that TNF-A levels were highest in the 10–19 age group, while the lowest levels were observed in patients aged 60 years and above. Similarly, IL-6 showed elevated levels in the 10–19 age group (42.14), while the lowest level was recorded at 22.52 in patients aged 60 years and above, although these differences were not statistically significant. In contrast, IL-32 levels remained relatively stable (ranging between 145–150) until the 50–59 age group, then increased sharply to 232.45 in those aged ≥60, without reaching statistical significance, as illustrated in Table 3.

When comparing the mean levels of TNF-A, IL-6 and IL-32 between rheumatoid arthritis patients and healthy controls, the study found higher levels in patients 37.19, 33.31 and 159.60 respectively) compared to controls (34.23, 22.37 and 134.24), as shown in Figure 1.

Regarding the distribution of TNF-A, IL-6 and IL-32 by gender, cytokine levels were found to be higher in male patients compared to females, as shown in Figure 2.

DISCUSSION

The study investigated the serum concentration of the pro-inflammatory cytokines TNF-α, IL-6 and IL-32 in patients with rheumatoid arthritis (RA) and estimated their distribution according to sex and age. Although higher mean cytokine concentrations were found in patients with RA than in healthy control groups, none of the observed differences reached statistical significance. Therefore, the findings should be interpreted with caution and regarded as descriptive trends rather than definitive associations.

The majority of patients were between the ages of 30 and 39, according to the study population's age distribution; however, this difference was not statistically significant (p = 0.19). This finding is mostly in line with epidemiological research showing that rheumatoid arthritis typically manifests in the fourth and fifth decades of life. According to Alamanos *et al.*, age-related immunological and hormonal changes that enhance vulnerability to autoimmune disorders may be the reason why the incidence of RA peaks at age 45. However, these results should be viewed as descriptive observations rather than proof of an age-related effect because the current investigation did not identify any statistically significant correlation.

In terms of age-specific cytokine levels, younger individuals (10–19 years) had the greatest mean concentrations of TNF-α and IL-6, while older age groups showed gradually lower values. These differences are consistent with other results indicating that younger people often show higher inflammatory responses due to more active innate and adaptive immune functions, even if they were not statistically significant (p>0.05) [13,14]. On the other hand, immunosenescence and persistent low-grade inflammation (also known as "inflammaging") are linked to aging and change immune modulation and cytokine production [13,14]. Larger study populations are needed for confirmation of these findings because statistical significance was not reached in this investigation.

In contrast to TNF-α and IL-6, IL-32 showed a higher mean concentration among individuals over 60, although it remained reasonably steady throughout most age groups. IL-32 has emerged as a key regulator of chronic inflammatory responses, thus even though this rise was likewise not statistically significant (p = 0.24), it merits more research. Through the activation of NF-κB and p38 MAPK signalling pathways, IL-32 stimulates the production of TNF-α, IL-1β and IL-6, which in turn promotes synovial inflammation, osteoclast activation and joint deterioration, according to experimental studies [15,16]. Additionally, synovial tissues from rheumatoid arthritis patients have been shown to express more IL-32, indicating that this cytokine may be involved in the development of the disease and ongoing inflammatory activity [15,16]. However, these biological pathways should be considered potential explanations rather than verified findings because the current investigation did not show statistically significant differences.

TNF-α, IL-6 and IL-32 mean serum concentrations were generally greater in rheumatoid arthritis patients than in

healthy controls, although none of these changes were statistically significant. TNF-α and IL-6 are still important therapeutic targets in the treatment of rheumatoid arthritis since prior research has repeatedly shown them to be important mediators of synovial inflammation and cartilage degradation [17]. In a similar vein, it has been shown that IL-32 is an upstream inflammatory cytokine that can enhance inflammatory cascades by increasing the production of TNF-α and IL-6 [18]. However, inconsistent results of circulating cytokine concentrations have been reported in a number of published research; some have shown considerable elevations, while others have found weak or non-significant differences. Differences in sample size, disease duration, disease activity, treatment status, laboratory techniques, ethnicity and study design may all contribute to these disparities.

Male patients had greater mean cytokine concentrations than female patients, according to the current study; however, these differences were not statistically significant ($p>0.05$). Therefore, the current findings do not allow for the definitive establishment of a relationship between sex and cytokine concentrations. According to earlier research, sex hormones may affect inflammatory reactions, with oestrogen and testosterone having distinct immunomodulatory effects [19]. Hormonal influences are still hypothetical and should be treated with caution because hormone concentrations were not assessed in this investigation.

The possible impact of anti-rheumatic treatment on cytokine concentrations is another crucial aspect that needs to be taken into account.

It has been demonstrated that biological treatments, especially TNF inhibitors and IL-6 receptor antagonists and disease-modifying anti-rheumatic medications (DMARDs) lower levels of inflammatory cytokines in the blood. The lack of data on treatment history, disease activity (DAS28) and length of illness in this investigation made it impossible to assess their possible impacts on cytokine concentrations, which may have added to the observed variability.

There are various limitations to the current investigation. First, the statistical power to identify meaningful differences between groups may have been diminished by the very small sample size. Second, despite their known impact on inflammatory biomarkers, treatment history, DAS28 score and length of illness were not documented. Third, it is difficult to determine causal linkages due to the cross-sectional design. To further understand the clinical importance of TNF-α, IL-6 and IL-32 in rheumatoid arthritis, future research with bigger cohorts, thorough clinical characterization and longitudinal follow-up is advised.

CONCLUSIONS

One of the most common diseases is rheumatoid arthritis, which is prevalent among those in their thirties in both sexes. The pro-inflammatory cytokine response of TNF-α and IL-6 was high in the younger age groups participating in the study, while the IL-32 response was higher in the older age groups.

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