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Evaluation Tumor Necrosis Factor-α (TNF- α) Levels in Patients with Rheumatoid Arthritis

Ali S. Shakir*

Abstract

Background: RA is an inflammatory, long-term autoimmune illness, that is impacted by external variables, according to a report from the World Health Organization. Women are 2-3 times more likely than men to develop RA, though this difference diminishes with older age at onset. Aim: The purpose of this study is to assess the significance of TNF-α concentrations in the predisposition to rheumatoid arthritis. Methods: The research project enrolled 100 patients diagnosed with rheumatoid arthritis (RA), admitted to the hospital from March to August 2023. These patients were compared with a control group consisting of 100 apparently healthy individuals. Each participant in both groups donated 3 milliliters of blood for analysis using the TNF-α ELISA (Mabtech USA) Kit. The study aimed to assess and compare TNF-α levels between RA patients and healthy controls, contributing to the understanding of TNF-α's role in RA pathogenesis and potential diagnostic implications. Results: The levels of TNF-α in the serum of patients with RA were significantly higher than those observed in the healthy control group. Conclusion: Significant association between TNF-α and RA.

Keywords: Rheumatoid arthritis, TNF-α, ELISA, disease predisposition, autoimmunity, pathogenesis, immunology.

INTRODUCTION

Rheumatoid arthritis is an inflammatory, long-term autoimmune illness, that is impacted by external variables, according to a report from the World Health Organization. In addition to contributing to functional disability, between 0.5% and 1.0% of people have RA [1]. The gender gap narrows with age at the onset of RA. However, women still have a 2-3 times greater prevalence rate than males. Although joint degeneration affects both sexes equally, women often experience poorer disease activity and disability outcomes than men [2]. Its main target is the synovial lining tissue, and it has the potential to bring about long-term disability as well as societal and economic costs [3]. It is challenging to diagnose RA at an early stage because there is currently no surefire test for the disease. Anticitrullinated protein-antibody (ACPA) and rheumatoid factor (RF) represent two of the most common autoantibodies in RA. When it comes to predicting the severity and course of RA, ACPA is reasonably predictive. Erosive RA can exist in patients without ACPA or RF, which affects 20-30% of the population [4]. For the purpose of understanding the processes involved in RA and to improve early detection and treatment of patients, further biomarkers are needed, even though RF and ACPA are useful for diagnosis [5]. Among autoimmune diseases, cytokines play a significant role; rheumatoid arthritis (RA) is the most well-known, affecting the synovial joints [6]. It is possible to trigger the autoimmune response by increasing the production of cytokines that promote inflammation. Pathogenic cells have been activated, differentiated, and migrated to the joints by these soluble substances, leading to injury and the activation of osteoclasts [7]. When it came to pathophysiology and treatment of rheumatoid

***Author for Correspondence**
Ali S. Shakir
E-mail: alihas@yahoo.com

Lecturer, Department of Clinical Immunology, College of Dentistry, University of Al-Qadisiyah, Diwaniyah, Iraq

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arthritis in 1985, cytokine expression was first mentioned. By 1988, tumour necrosis factor (TNF) had been established as a potent cytokine with a wide range of effects, the most well-studied of which was its capacity to promote inflammation [8]. A high level of inflammatory cytokines, rather than inhibitory ones, is responsible for the characteristic inflammation seen in RA [9]. One of the most harmful pro-inflammatory cytokines, tumour necrosis factor (TNF-α), controls the release of prion flammatory chemo kines in synovial tissue. Joint damage and dysfunction can result from TNF-α and IL-1β pro-inflammatory characteristics, their capacity to activate T cells, and the release of other chemicals having comparable effects [10].

Unlike in synovial fluid, where CD8+T cells predominate, data indicate that CD4+T cells are the most prevalent infiltrating cells in the synovial membrane. However, these cells are primarily responsible for causing, maintaining, and exacerbating chronic inflammation [11]. Antagonized CD4+T cells recruit monocytes, macrophages, and synovial fibroblasts through the TCR. This stimulus promotes the synthesis of the cytokines IL-1, IL-6, and TNF-α, which are the main inflammatory mediators in RA, according to Choy and Panayi [12]. Initiating the disease is most likely caused by TNF- α and IL-1[13].

MATERIALS AND METHODS

Study Design

The case-control design was used to examine the following groups of participants. There were two groups of subjects that participated in this research. One hundred patients were diagnosed with rheumatoid arthritis in the first group. For the purpose of this study's control group, 100 volunteers who appeared to be in good health and of similar age were graciously recruited. We did not include patients who smoked or used alcohol.

Sampling Criteria

Veins were punctured using disposable syringes to obtain five milliliters' (ml) of blood in an aseptic manner. The 3 ml of venous blood was collected into tubes that separated serum using separating gel. These tubes were centrifuge at 2000-3000 rpm for 5 minutes to extract the serum, then tested for TNF-α using an ELISA.

Statistical Analysis

The data was characterised, analysed, and presented using SPSS version 22, a social science statistical Programme. Both means and standard deviations (SD) were employed for the quantitative variables' evaluation. We used percentages and frequencies to quantify the qualitative features. An independent T-test was used to compare the mean values of the two groups. Through the application of Pearson's correlation, two quantitative variables were connected. The significance level of $P \leq 0.05$ was determined by researchers.

RESULTS

Characteristics of the Study Population

100 RA patients and 100 healthy controls participated in the current research. Table 1 displays the demographic information of both the patients and the control participants. The age distribution of the participants was as follows: 48.75 ± 12.03 years for RA patients and 43.90 ± 10.43 years for control subjects. No-statistically significant variation was found (P = 0.129) between the two groups. Enrollment was 167 girls (83.5%) and 33 males (16.5%). With 13 male cases (13.1%) and 87 female cases (87.0%) included in the RA patients and 20 male cases (20.0%) included in the control subjects, there was non-statistically significant variation in the frequency distribution of both group regarding gender (P = 0.262). The sick group's body mass index (BMI) was found to be somewhat higher (28.90 ± 3.52) than the control group's (27.13 ± 3.03), although non- significant variation ($p < 0.05$). In contrast to the control groups, individuals with RA had a significantly higher ESR (27.68 ± 7.77) compared to (12.80± 2.38).

Table 1. Characteristics of patients with hemodialysis and control.

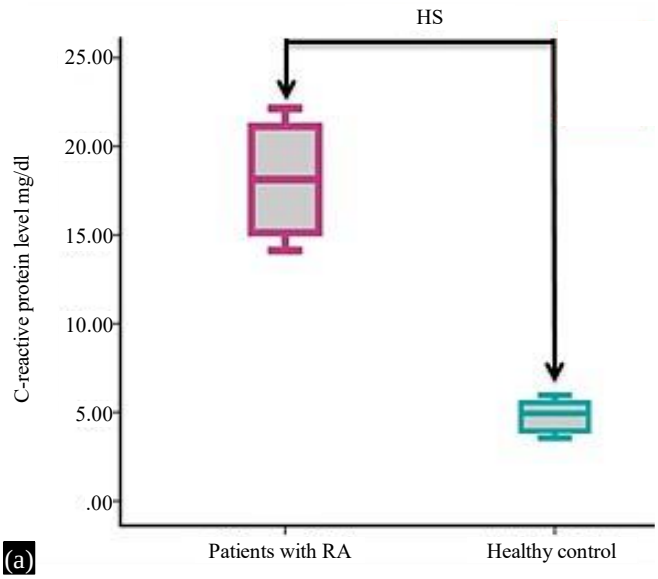
Characteristics	Patient (n=100)	Control (n=100)	*P*
Age	48.75 ± 12.03	43.90 ± 10.43	0.129
Gender			
Male, *n (%)*	13 (13.0 %)	20 (20.0%)	0.262
Female, *n (%)*	87 (87.0%)	80 (80.0%)	
BMI kg/m2	28.90 ± 3.52	27.13 ± 3.03	0.129
ESR	27.68 ± 7.77	12.80± 2.38	< 0.001
Rheumatoid factor (RF)			
Positive, *n (%)*	28 (56.0%)	0	< 0.001
Negative, *n (%)*	22 (44.0%)	100 (100.0%)	
CRP mg/dl	18.13 ± 4.44	4.95 ± 1.13	< 0.001
Anti-CCP mg/dl	55.29 ± 7.22	5.85± 1.47	< 0.001
TNF-α ng/dl	2.55 ± 0.311	1.05 ± 0.282	< 0.001

Measurements of inflammatory parameters

Figure 1 C-reactive protein concentrations in patients with RA were highly significantly higher than healthy control subjects (18.13 ± 4.44 mg/dl vs 4.95 ± 1.13 mg/dl, respectively, P< 0.001). Furthermore, erythrocyte sedimentation rate (ESR) levels in patients with RA were significantly higher than healthy control subjects (27.68 ± 7.77 mm/h vs 12.80± 2.38 mm/h, respectively, P< 0.001). Regarding to Anti-cyclic citrullinated peptide (Anti-CCP), Anti-CCP concentrations in patients with RAs was significantly greater than healthy control subjects (55.29 ± 7.22 mg/dl and 15.85± 1.47 mg/dl, respectively, P < 0.001). Patients with RA have positive Rheumatoid factor (RF) accounted for 28 (56.0%), while no positive rate in control group (0 %) and the variation was highly significant (p= 0.001).

Diagnostic accuracy of Tumor Necrosis Factor-α

Figure 2 and Table 2 We used receiver operating characteristic (ROC) analysis to find out how well TNF-α level could differentiate between patients with rheumatoid arthritis and healthy controls. A sensitivity of 80.0%, specificity of 70.0%, PPV of 72.7%, and NPV of 77.8% were achieved with an ideal TNF-α cut-off value of 1.81 ng/ml, leading to an AUC value of 0.731 (95% confidence interval [CI], 0.625-0.836, P < 0.001).



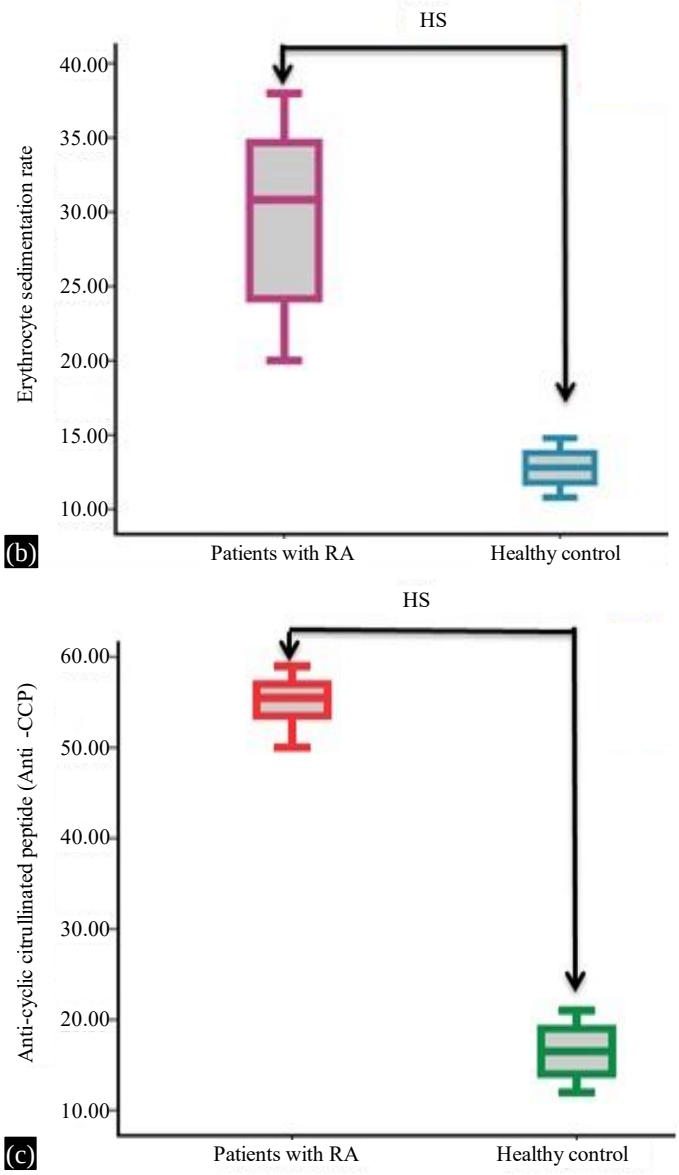


Figure 1. (A) C-reactive protein (CRP) concentrations in healthy control volunteers and patients with RA. (B) Rate of erythrocyte sedimentation in participants with RA and healthy controls. The anti-cyclic citrullinated peptide (Anti-CCP) was studied in both RA patients and healthy controls. The results are extremely significant ($P < 0.001$) according to the HS assessment.

Table 2. Roc curve of TNF-α level.

Characteristic	TNF-α level
Cutoff value	> 1.81
P value	< **0.001**
Sensitivity %	80.0 %
Specificity %	70.0%
PPV %	72.7 %
NPV %	77.8%
AUC (95% CI)	0.731 (0.625- 0.836)

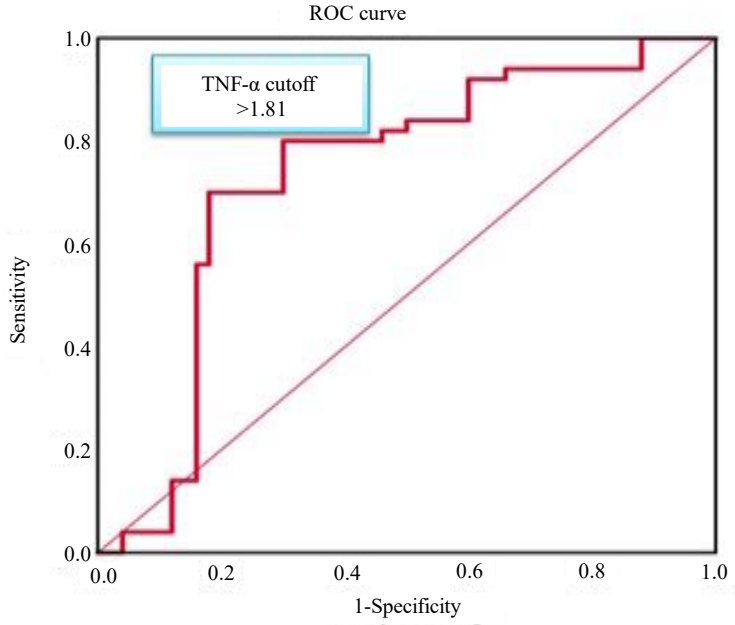


Figure 2. ROC Analysis of TNF-α for the purpose of calculating a potential diagnostic cutoff value.

Table 3. Correlation between TNF-α and inflammatory markers (CRP, RF, ESR and anti-CCP).

Characteristic	*TNF-α*	
	r	***P***
CRP	0.294	0.018*
RF	0.200	0.164
ESR	0.064	0.658
anti-CCP	0.347	0.001*

r: correlation coefficient.

Correlation Between TNF-α and Inflammatory Markers (CR-P, RF, ESR and Anti-CCP)

Data showed the association between TNF-α and inflammatory markers, including CR-P, R Factor, ESR, and anti-CCP. Data indicate a strong positive relationship between TNF-α and CRP (r=0.294, p=0.018) and TNF-α and CRP (r=0.347, p=0.001) in individuals with RA. Correlations between other parameters are not statistically significant in the other results.

DISCUSSION

The results showed there was nonsignificant difference between the healthy controls and patients with respect to gender, age, or body mass index [14]. The ESR and CRP are two widely used indicators of inflammation in RA, where the obtained results indicate that there are significant differences between the two groups of patients and control, so an increase in sedimentation rate indicates the presence and increase of inflammation in rheumatoid arthritis patients. The findings are in line with those of the study by **Özsoy** *et al.,[15],* which found that a very high ESR is still a significant clinical indicator and that rheumatic causes, in addition to cancer and infections, are substantial. High C-reactive protein levels in the blood suggest chronic inflammation, and the results demonstrated that these levels were increasing in RA patient versus healthy participant. This is in line with the findings of the study by Janet and Pope, which showed that C-reactive protein (CRP) is an essential regulator of systemic inflammation in rheumatoid arthritis (RA) and seems to have a direct impact on bone loss and radiographic advancement. A etiology of frequent RA comorbidities has also been linked to CRP [16].

Additionally, the data demonstrated that the rheumatoid factor was significantly different in the comparisons between the patient and healthy group. Autoimmune disorders, including rheumatoid arthritis, are commonly associated with elevated blood RF levels. Evidence suggests that immunological responses to certain pathogenic organisms rely on low-affinity RFs. In contrast, high-affinity RF. RF are a sign of a more severe and long-lasting RA condition in patients. RFs may regulate Ig synthesis by modulating B cell activation; their effects on this process may vary by genetic background, but they are likely an immunological response product of inflammation [17]. In addition, compared to healthy controls, RA patients had significantly higher levels of antibodies to cyclic citrullinated peptide (anti-CCP). Anti-CCP is a more specific diagnostic tool for RA than rheumatoid factor (RF) [18]. This is because TNF-α activates synovial fibroblasts, leading to an excess of cathepsins and MMP, and the study found that the sick group had much higher TNF-α values than the healthy group. Joint erosion and the subsequent deterioration of cartilage and bone are consequences of the proteoglycan and collagen breakdown that follows. Synovial hyperplasia and angiogenesis are caused by osteoclasts in RA [19-24].

CONCLUSIONS

Autoimmune conditions, containing rheumatoid arthritis, are ordinarily supplementary with elevated blood RF levels. Evidence suggests that immunological responses to certain pathogenic organisms rely on low-affinity RFs. In contrast, high-affinity RF. RF are a sign of a more severe and long-lasting RA condition in patients. RFs may regulate Ig synthesis by modulating B cell activation; their effects on this process may vary by genetic background, but they are likely an immunological response product of inflammation

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