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Research Article

Interleukin-6 Suppression by Different TNF Inhibitors in Rheumatoid Arthritis Patients During Maintenance Therapy

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Abstract

Background: Inhibiting TNF-α may indirectly impact IL-6 production and reduce the overall inflammatory response in rheumatoid arthritis (RA). ***Objectives***: To evaluate the effect of different TNF inhibitors on plasma IL-6 levels and disease activity in RA patients. ***Methods***: A longitudinal, observational study included 65 adult RA patients receiving TNF inhibitors for at least six weeks in Sulaymaniyah, Iraq, between February and August 2022. Disease activity was assessed using the disease activity score 28 (DAS28). Plasma IL-6 levels, CRP, and ESR were measured at two time points over 14 weeks during maintenance therapy. ***Results***: After 14 weeks, adalimumab lowered IL-6 levels to 31.92 ng/L (0.24), ESR to 15 mm/hr (16.8), and CRP to 8.4 mg/L (26.2). DAS28/ESR was also improved, it went from 4.7 (1.2) to 4.0 (1.3), which was the biggest change. Infliximab decreased IL-6 31.87 ng/L (0.29), ESR 10.1 mm/hr (15.0), and CRP 7 mg/L (13.5), but the smallest improvement was in DAS28/ESR 4.6 (1.6) to 4.5 (1.2). Etanercept exhibited the lowest decrease in IL-6, ESR, and CRP: 31.77 ng/L (0.39), 7.5 mm/hr (6.5), and 4.9 mg/L (4.9), respectively. However, it showed the second-greatest median improvement in DAS28/ESR, from 4·4 (1·8) to 4·0 (1·4). No correlation was found between plasma IL-6 levels and DAS28/ESR at either time point. ***Conclusion***: TNF inhibitors differ in reducing plasma IL-6 levels and DAS28 during maintenance therapy in RA patients. Adalimumab was the most effective in reducing IL-6, CRP, ESR levels, and DAS28.

Keywords: Biological therapy, DAS28, Interleukin-6, Maintenance therapy, Rheumatoid arthritis, TNF inhibitors.

قمع إنترلوكين -6 بواسطة مثبطات عامل نخر الورم المختلفة في مرضى التهاب المفاصل الروماتويدي أثناء علاج المداومة

الخلاصة

الخلفية: قد يؤثر تثبيط عامل نخر الورم - α بشكل غير مباشر على إنتاج IL-6 ويقلل من الاستجابة الالتهابية الكلية في التهاب المفاصل الروماتويدي (RA). **الأهداف**: تقييم تأثير مثبطات عامل نخر الورم المختلفة على مستويات IL-6 في البلازما ونشاط المرض لدى مرضى التهاب المفاصل الروماتويدي. **الطريقة**: شملت دراسة طولية قائمة على الملاحظة 65 مريضا بالغا بالتهاب المفاصل الروماتويدي يتلقون مثبطات عامل نخر الورم لمدة ستة أسابيع على الأقل في السليمانية، العراق، بين فبراير وأغسطس 2022. تم تقييم نشاط المرض باستخدام درجة نشاط المرض (DAS28) وتم قياس مستويات IL-6 في البلازما و CRP و ESR في نقطتين زمنيتين على مدار 14 أسبوعا أثناء العلاج الوقائي. **النتائج**: بعد 14 أسبوعا، خفض أداليموماب مستويات IL-6 إلى 31.92 نانوغرام/لتر، ESR إلى 15 ملم/ساعة ، وCRP إلى 8.4 ملغ/لتر. كما تحسنت DAS28 / ESR، حيث انتقلت من 4.7 إلى 4.0، وهو أكبر تغيير. خفض انفليكسماب IL-6 31.87 الى 31.87 نانوغرام/لتر، و ESR الى 10.1 مم/ساعة ، و CRP الى 7 مجم/لتر، ولكن أصغر تحسن كان في DAS28/ESR الى. أظهر Etanercept أدنى انخفاض في IL-6 و ESR و CRP الى 31.77 نانوغرام/لتر، 7.5 مم/ساعة، و 4.9 مجم/لتر، على التوالي. ومع ذلك ، فقد أظهر ثاني أكبر تحسن متوسط في DAS28 / ESR، من 4.4 الى 4.0. لم يتم العثور على ارتباط بين مستويات IL-6 في البلازما و DAS28 / ESR في أي من النقطتين الزمنيتين. **الأستنتاج**: تختلف مثبطات عامل نخر الورم في تقليل مستويات IL-6 في البلازما و DAS28 أثناء علاج المداومة في مرضى التهاب المفاصل الروماتويدي. كان أداليموماب الأكثر فعالية في تقليل مستويات IL-6 و CRP و ESR و DAS28.

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease [1], characterized by overexpression of the pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) as a result of interactions between T and B lymphocytes, synovial fibroblasts, and macrophages, causing chronic synovial inflammation and joint destruction with debilitating effects [2,3]. One of the characteristics of RA is the synovial fibroblasts' continued synthesis of IL-6 after TNF-stimulation [4]. Although TNF-α and IL-6 have overlapping and synergistic effects, some of their effects are regulated by separate pathways. As a result, it is critical to block the activity of cytokine variants, which can play a key role in the development of inflammation, tissue degradation, and auto-antibody specificities in RA patients. Several studies have investigated the role of TNF-α and IL-6 in RA. A study found that IL-6 signaling attenuates TNF-α production in rheumatoid arthritis [5]. IL-6 is the most often expressed cytokine in RA patients and plays multiple roles in the etiology of RA [6]. IL-6 stimulates the production of acute-phase proteins such as C-reactive protein (CRP), fibrinogen, haptoglobin, and serum amyloid-A and the proliferation and differentiation of osteoclasts; therefore, IL-6 induces bone resorption, and its levels correspond with disease activity and joint destruction [6,7]. CRP and erythrocyte sedimentation rate (ESR) are markers of inflammation despite not being RA-specific; they serve as a diagnostic tool for the disease and are also used to track patients' levels of inflammation while they are receiving treatment [8], and a high level is linked to radiographic progression [6]. The goal of disease-modifying anti-rheumatic drugs (DMARDs), which slow down the damage to joints and reduce inflammation, is to improve how well the body works. DMARDs include traditional conventional synthetic DMARDs (csDMARDs) (methotrexate, hydroxychloroquine, and sulfadiazine), targeted synthetic DMARDs (pan-JAK- and JAK1/2-inhibitors), and biologic DMARDs (bDAMRDs), including TNF-α inhibitors, TNF-receptor inhibitors, IL-6 inhibitors, IL-6R inhibitors, and B cell-depleting antibodies [9]. While TNF inhibitors are primarily employed to block the function of TNF-α, they may also affect IL-6 levels in RA patients. However, the precise nature of this effect may depend on multiple factors, particularly the specific type of inhibitor used and the markers of inflammation being studied. Therefore, the present study aimed to evaluate the effect of different TNF inhibitors on reducing plasma IL-6 levels and other inflammatory markers and to assess the relationship between plasma IL-6 levels and disease activity based on DAS28/ESR in RA patients.

METHODS

A longitudinal, observational study with 65 adult RA patients (48 women and 17 men) receiving TNF inhibitor treatment was carried out at the Center for Rehabilitation and Rheumatology in Sulaimani City, Iraq, from February to August 2022. Adults ≥18 years who have been diagnosed with RA using the 2010 American College of Rheumatology/European Alliance of Associations for Rheumatology (ACR/(EULAR) guidelines,[10] and who started any of the three TNF inhibitor therapies (infliximab, adalimumab, or etanercept) within the last six weeks(maintenance phase) were included in the study regardless of their disease activity and concurrent medications. Patients with demyelinating disease, tuberculosis (TB), Hepatitis B or C, pregnancy or lactation, heart failure, and prior or contemporaneous malignancies were excluded (Figure 1).

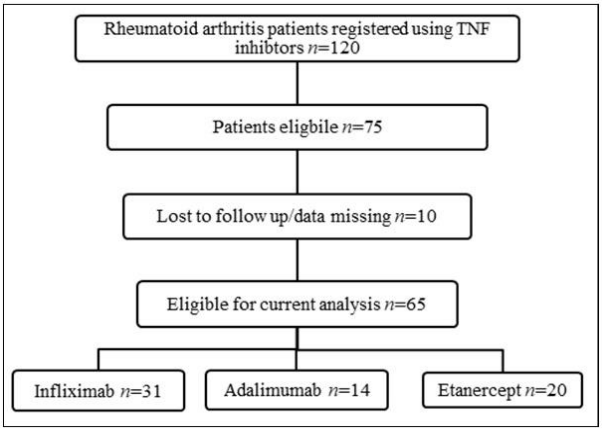

Figure 1: Flow chart of the study design.

Ethical consideration

The study protocol was approved by the Ethics Committee of the College of Pharmacy/University of Sulaimani under the registration number (PH32-21). The study was conducted in compliance with the Declaration of Helsinki's guiding principles and the most recent International Conference on Harmonization rules. Following oral and written information about the trial, all patients provided written informed consent. The study was registered in the clinical trials database (ClinicalTrials.gov Identifier: NCT05379049).

TNF inhibitors

Three TNF inhibitors (infliximab, adalimumab, and etanercept) with different dosing schedules were used in the current study. Infliximab 100 mg/IV (3 mg/kg) at weeks 0 and 2, adalimumab 40 mg/SC at weeks 0 and 4, and etanercept 50 mg/SC at weeks 0 and 1, 2, and 3 were all given throughout the induction phase. During the maintenance phase, adalimumab, infliximab, and etanercept were administered every

two weeks, every eight weeks, and weekly, respectively.

IL-6 assay

Before the patients received the next dose of the TNF inhibitor in the morning (8:30 am–11 am), venous blood samples were taken from them. Blood was drawn into test tubes containing the anticoagulant ethylene diamine tetraacetic acid (EDTA), mixed for 10 to 20 minutes, and then centrifuged for 20 minutes at 3000 rounds per minute (RPM). The resultant plasma was collected in Eppendorf tubes and kept chilled until analysis at -70 °C. Plasma levels of IL-6 were measured twice during a 14-week period using sandwich enzyme-linked immunosorbent assay (ELISA) kits (Bioassay Technology Laboratory, Korean Biotech Co., Ltd.). The reference range was 2 ng/L to 600 ng/L, and the analytical sensitivity was 1.03 ng/l. According to the manufacturer's method, IL-6 from the sample was added to the wells. This causes the human IL-6 antibodies coated in the wells to interact with each other. The streptavidin-HRP, which binds to the biotinylated IL-6 antibody, was then added. After incubation, plate wells were cleaned. By adding the substrate solution and monitoring the resulting color, the concentration of human IL-6 was adjusted. The reaction was stopped once a stop solution was added, and after 10 minutes, absorbance at 450 nm was measured. We developed the linear regression equation of the standard curve, which is distinct for the parameter, based on standard concentrations and related optical density (OD) values. The concentration of the matching sample was then determined based on the OD value of the samples. Also, acute phase reactants ESR and CRP were measured at two time points spaced by 14 weeks, and the results were provided by the Rehabilitation and Rheumatology Center.

Clinical assessment based on DAS28/ESR

We made a composite measure (called DAS28/ESR) that uses the patient global health assessment (PtGA), the tenderness and swelling of 28 joints, and the inflammatory marker ESR to evaluate the disease activity in RA [11]. The following formula was used to determine the scores at two time points that were separated by 14 weeks:

$$DAS28/ESR = 0.56 \sqrt{(TJC28)} + 0.28 \sqrt{(SJC28)} + 0.70 \ln(ESR) + 0.014 (GH)$$

TJC is the tender joint count, SJC is the swollen joint count, GH is the patient's global health assessment (VAS), and ESR is the erythrocyte sedimentation rate. According to the DAS28/ESR score, the patients were divided into four stages of the disease: remission (DAS28 < 2·6), low disease activity (DAS28≥2·6 and ≤3·2), moderate disease activity (DAS28 >3·2 and ≤5·1), and high disease activity (DAS28 >5·1).

Statistical analysis

SPSS version 23 (IBM SPSS Statistical Package for the Social Sciences) was used for the statistical analysis, and the figures were created using GraphPad Prism 9. In order to describe non-normally distributed quantitative variables like IL-6 and DAS28, the median and interquartile ranges (IQR) were utilized. The nonparametric Wilcoxon sign rank test was used to compare the medians (IQR) of the three treatment groups, while the Kruskal-Wallis test was used to compare the medians (IQR) of each treatment group between the two time periods. The threshold for statistical tests' significance was set at *p*-values less than 0.05.

RESULTS

According to the patient demographics, females showed a 2.8-fold higher prevalence of the disease compared to males. The average disease duration was 9.9 years, with the majority of patients (61%) having a disease duration of ≤10 years. Regarding biological therapy, the average duration was 19·033±28·6 months, with the greatest number of cases (56.9%) lasting less than six months and the lowest percentage (15.6%) lasting more than 36 months. (Table 1). The median (IQR) changes in CRP and ESR levels at two points in time 14 weeks apart for the TNF inhibitor maintenance therapy were not statistically significant (Table 2 and Figure 2).

Table 1: Demographic characteristics of RA patients using TNF inhibitors

Variables	Mean±SD	Groups	*n*(%) Patients
Gender		Male	17 (26.2%)
		Female	48 (73.8%)
Weight (kg)	75.1±15.4	All	65 (100)
Age (Years)	48.6±13.8	19-29	6 (9.2%)
		30-39	10 (15.4%)
		40-49	18 (27.7%)
		50-59	16 (24.6%)
		≥ 60	15 (23.1%)
Disease duration (year)	9.9±7.0	≤ 5	20 (30.8%)
		6-10	20 (30.8%)
		11-15	9 (13.8%)
		≥ 16	16 (24.6%)
TNF inhibitors		Infliximab	31(47.69%)
		Adalimumab	14 (21.54)
		Etanercept	20 (30.77)
TNF inhibitors therapy duration (month)	19.03±28.6	1.5 < x < 6	37 (56.92%)
		7-12	7 (10.76%)
		13-36	11(16.92%)
		≥ 37	10 (15.38%)

n (%): number and percentage of patients.

Adalimumab showed the largest decreases in CRP (8.4 mg/L) and ESR (15.0 mm/hr) compared to the other drugs. Even though the median (IQR) plasma IL-6 level dropped significantly between the two time points for each TNF inhibitor, adalimumab had the biggest drop at 31.83 ng/L (0.35), followed by

infliximab at 31.8 ng/L (0.24) and etanercept at 31.77 ng/L (0.37). However, there was no significant difference between the various TNF inhibitors (Table 3).

Table 2: Change in median (IQR) CRP and ESR levels between two time points spaced by 14 weeks in RA patients maintained on TNF inhibitors

Change in median (IQR) between two time points	Infliximab (*n*=31)	Adalimumab (*n*=14)	Etanercept (*n*=20)	*p*-value
CRP (mg/L)	7 (13.5)	8.4 (26.2)	4.9 (4.9)	0.30
ESR (mm/hr)	10.1 (15.0)	15 (16.8)	7.5 (6.5)	0.15

n: number of patients.

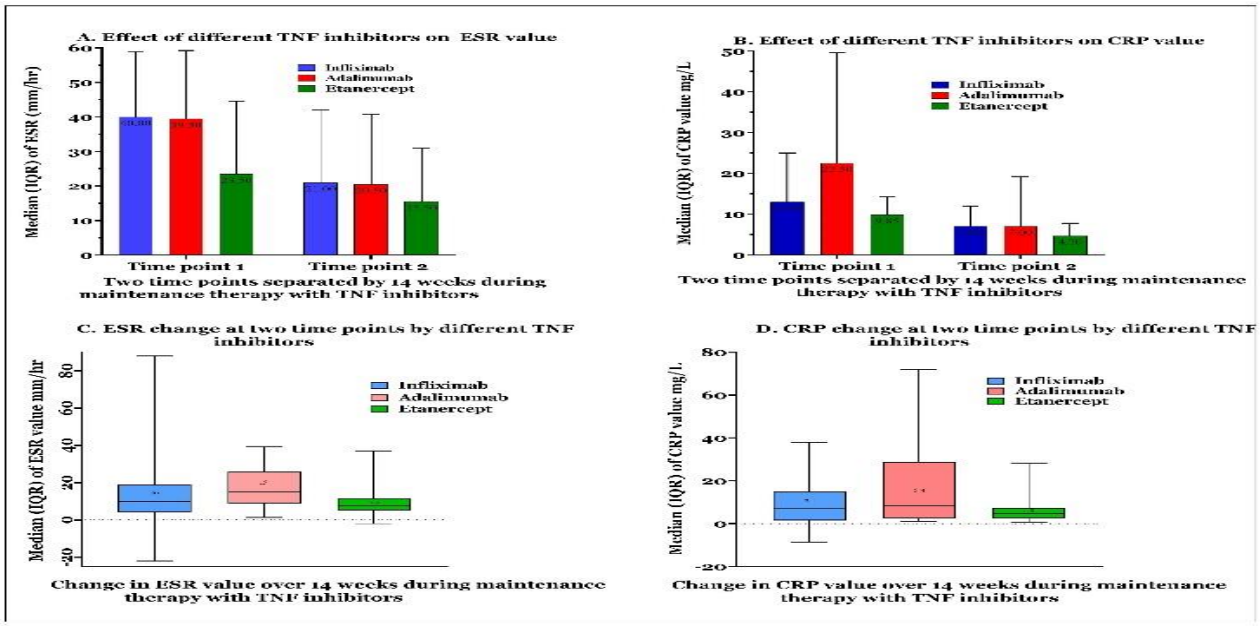

Figure 2: Effects of infliximab, adalimumab, or etanercept on the ESR (A & B) and CRP (C & D) at two time points separated by 14 weeks in RA patients maintained on TNF inhibitor therapy.

Table 3: Effect of different TNF inhibitors during maintenance therapy on the plasma IL-6 (ng/L) median (IQR) between two time points (14 weeks) in RA patients

Time points	Infliximab (*n*=31)	Adalimumab (*n*=14)	Etanercept (*n*=20)	*p*-value
Time-point 1	91.21 (1.09)	91.36 (1.05)	91.19 (1.04)	0.94
Time-point 2	59.21 (0.80)	59.28 (1.22)	59.31 (0.93)	0.82
Change	31.8 (0.24)	31.83 (0.35)	31.77 (0.37)	
p-value	< 0.001	< 0.001	< 0.001	

n: number of patients.

Even though DAS28/ESR for all of the three TNF inhibitors declined within the two time points, adalimumab showed the greatest change in DAS28/ESR [4·7 (1.2) to 4·0 (1.3)], next came etanercept [4.4 (1.6) to 4.0 (1.2)], and infliximab showed the least change [4.6 (1.6) to 4.5 (1.2)] (Figure 3).

DISCUSSION

Since a cytokine network is involved in the pathogenesis of RA, inhibiting one cytokine, such as TNF-α, could affect other cytokines, especially IL-6, since it's one of the most commonly expressed cytokines in RA patients and has overlapping effects with TNF-α. So, in this study, we looked at the relationship between plasma IL-6 levels and disease activity in RA patients receiving maintenance therapy with different TNF inhibitors (infliximab, adalimumab, or etanercept) over 14 weeks, as well as other inflammatory biomarkers like CRP and ESR. Younger-onset rheumatoid arthritis (YORA), which develops before the age of 60, was validated by the findings of the current study. The patients' mean age was 48·6±13·8 years, with the age group of 40 to 49 years having the highest frequency, which is in line with the findings of earlier studies [12,13].

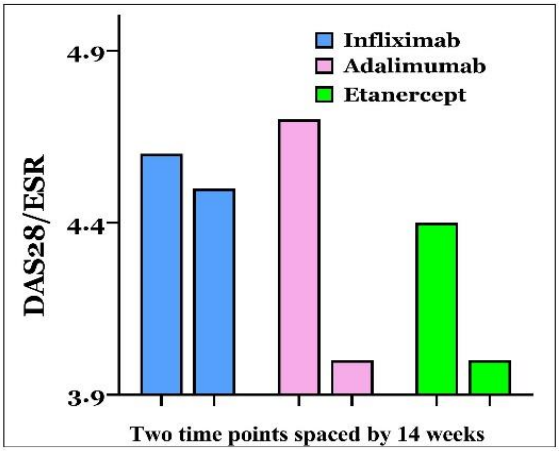

Figure 3: Change in the median (IQR) of DAS28/ESR between two time points during treatment with infliximab, adalimumab, or etanercept over a 14-week period in RA patients.

Table 4: Relationship between median (IQR) of plasma IL-6 levels (ng/L) and DAS28/ESR in RA patients maintained on infliximab, adalimumab, or etanercept at two time points over 14 weeks

Disease activity staging	Plasma IL-6 ng/L median (IQR)				
	n	Time-point 1	*n*	Time-point 2	*p*-value
Remission (DAS28<2.6)	1	92.77	2	59.36	NS
Low (DAS28≥2.6 and ≤3.2)	8	90.80 (0.49)	11	59.08 (0.51)	0.003
Moderate (DAS28>3.2 and≤5.1)	38	91.30 (1.02)	41	59.14 (0.76)	0.001
High (DAS28>5.1)	18	91.25 (0.99)	11	59.58 (1.04)	0.003
p-value		<0.09		<0.25	

n = number of patients.

The mean duration of the disease was 9.9±7.0 years, with the highest frequency being ≤10 years, which was consistent with the results of a study conducted in Turkey [14], and other studies with a mean duration of the disease of 9·32±7·26 [15], and 6·70±5·96 years [16]. Ramos *et al*. (2017), who carried out multicenter cohort research on 2481 patients (82% female), with a mean age of RA onset of 44±14 years, may help to explain YORA in the Mediterranean region. They observed that, in addition to the age factor, there may be a correlation between a number of criteria, such as pollution, infection exposure, and atmospheric features adjusted by geographic coordinates, all of which have been adjusted for inequality [17]. There is widespread recognition that, when it comes to gender risk factors, women are more likely than men to develop the condition. Similar to earlier research, the prevalence of the condition was 2·8:1 greater in females in the current study, despite various female-to-male ratios being observed, including 3.2:1 [12], 3.5:1 [18], 4:1 [13], and 4.7:1 [14]. The study's findings revealed that the patients were obese or overweight (Table 1). The body mass index (BMI) and weight have been linked to a higher likelihood of RA, and numerous studies have emphasized the significance of being overweight or obese as a separate risk factor for RA [19]. In the setting of immune-mediated inflammatory diseases, obesity has been associated with more severe disease activity, a worse quality of life, and an inadequate response to treatment [20]. Additionally, earlier research has indicated a relationship between TNF inhibitors and an increase in body weight and BMI and that the emergence of obesity is one probable side effect of TNF inhibitors [21]. It would be helpful for clinicians to know for sure whether the TNF inhibitor systematically results in an increase in weight as a side effect and how much weight gain may be anticipated over a given time frame [22]. The data presented showed that TNF inhibitors decrease ESR and CRP levels. This decrease in the levels of ESR and CRP comes in consistency with other studies performed for RA patients using TNF inhibitors [23,24], but at different levels when considering the three treatments, the highest median (IQR) change for ESR was recorded for adalimumab at 15.0 (16.8) mm/hr. Also, other studies showed adalimumab to decrease ESR the most. [25], [26] While the current study showed that adalimumab had the greatest decrease in CRP level, a study in France showed a slight difference between adalimumab and etanercept, with etanercept having a lower CRP level [27]. According to a study, adalimumab administration decreased the levels of the inflammatory mediators IL-6 and disease activity indices DAS28, ESR, and CRP. Since these mediators are produced by macrophages, a decrease in the chemerin-dependent accumulation of synovial macrophages could explain their decline. As a result, it is possible that chemerin, which is a chemokine that precisely regulates the chemotaxis and activation of dendritic cells and macrophages, it plays a unique role in the pathophysiology of RA by influencing macrophage migration and/or retention in the synovium [28]. Although ESR and CRP have been employed as markers of inflammation by physicians for many years, there is still no clear agreement regarding when to use one of them or both. Recently, CRP has surpassed other serological markers as the main indicator of acute disease activity [29]. However, there is contradictory evidence on the relative CRP and ESR values in the disease course [30]. A study revealed that neither CRP nor ESR is superior in the clinical context to monitor inflammatory activity in the RA patient and that the relevance of and dependence on ESR and CRP as markers of inflammation in RA patients in routine treatment should be re-evaluated [31]. Due to its pro-inflammatory activities, IL-6 plays a crucial role in

the onset and progression of RA. Certain studies performed in Iraq have stated that plasma IL-6 levels in RA patients are much higher when compared to healthy subjects, which could sometimes be undetectable [32,33]. This has been confirmed by other studies as well [34,35]. Therefore, in this study, IL-6 was measured at two time points. At the beginning of the study, there was no significant difference between the different treatments. Also, the results showed that IL-6 decreased significantly in the patients, with the highest decrease recoded for adalimumab, which comes in accordance with a previous study, and that this decline is most likely a result of the drug's ability to reduce inflammatory activity [36]. Other studies also support the association between IL-6 and disease activity, stating that IL-6 levels decrease when clinical symptoms improve after receiving different TNF inhibitor treatments [37]. There have not been many studies on the relationship between plasma IL-6 and DAS28/ESR in RA patients treated with different TNF inhibitor maintenance therapies. This study did not find significant differences in plasma IL-6 levels across the DAS28 states that there is statistically no significant correlation between plasma IL-6 and DAAS28/ESR [32]. However, the study found that higher DAS28 scores were associated with higher plasma IL-6 levels, which is consistent with the findings of another study [38]. A positive correlation between the plasma level of IL-6 and disease activity based on DAS28 in RA patients has been found by Milman (2010) [38]. Additionally, this study found that plasma IL-6 decreased after 14 weeks at each disease stage, suggesting this decrease in plasma IL-6 as an indicator for patients shifting back at the disease stages towards remission or low disease activity. Therefore, in the future, IL-6 might be a clinically helpful early indicator of therapy response and disease progression, but further studies are needed to fully understand its function. In the present study, more than half of the RA patients had a disease duration of 10 years or less instead of being newly diagnosed patients, while the remaining patients had a disease duration of more than 10 years. This might explain the nonlinear relationship between IL-6 and DAS28. Following 14 weeks of treatment with various TNF inhibitors, 10% of the patients experienced a change from high to moderate disease activity, followed by a change from moderate to low disease activity. This is consistent with past research that found TNF-inhibitor-using RA patients' DAS28/ESR decreased over time [14]. TNF inhibitors have been shown in numerous studies to dramatically reduce the disease activity of RA patients, as measured by clinical scores like DAS28 and ESR. The results of the study demonstrated that patients in the etanercept and adalimumab groups responded to these therapies with higher rates than those in the infliximab group, despite similar median DAS28 values across treatment groups [39]. However, this is consistent with past studies that indicated adalimumab users had decreased disease activity. Adalimumab has been demonstrated to be more effective than etanercept and infliximab in reducing disease activity [23]. But, as is also supported by other research, there was no statistically significant difference between the three treatments [25]. Etanercept and adalimumab both decreased DAS28/ESR, although etanercept patients had the greatest decrease in disease activity, according to Greenberg *et al*. (2012) [39]. Contrarily, compared to etanercept, adalimumab and infliximab both had lower frequencies of low disease activity and prolonged remission, which is consistent with the current study's findings that infliximab only marginally decreases DAS28. The variance in responses to various TNF inhibitors may result from a number of variables, including patient demographics, disease activity and duration, and joint dysfunction [40]. It's essential to state that this study was observational and lacked a control group. The results of this study need to be confirmed by further research in order to identify the variables that predict clinical response as well as the most effective method to employ TNF inhibitors for RA patients in the maintenance phase.

Conclusion

The results showed that RA patients' plasma levels of IL-6 could be used to predict whether or not TNF inhibitors will work in the maintenance phase of treatment. Adalimumab was the most effective TNF inhibitor in reducing IL-6 levels, CRP, ESR, and DAS28. Infliximab and etanercept reduced IL-6 levels but were not as effective as adalimumab in reducing CRP, ESR, and DAS28.

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Conflicts of interest

There are no conflicts of interest.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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