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Article

The plasma Tumor Necrosis Factor-α (TNF-α) does not have any correlation with disease activity in rheumatoid arthritis patients treated with disease modifying anti-rheumatic drugs (DMARDs)

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We performed this study to measure the Tumor Necrosis Factor-alpha (TNF-α) plasma level and to survey its correlation with disease activity in the newly diagnosed Rheumatoid Arthritis (RA) patients and those who were under treatment with the combination of Disease-Modifying Anti-Rheumatic Drug (DMARD) plus Prednisolone (PSL).We enrolled 30 newly diagnosed RA patients who received no treatment regarding their disease, 30 patients under treatment with the combination of Methotrexate (MTX) + Hydroxychloroquine (HCQ) + PSL and 30 healthy subjects in this case-control study from September 2017 to December 2017. The level of plasma TNF-α was measured by enzyme-linked immunosorbent assay (ELISA) in each group. For assessment of disease severity, we used Disease Activity Score-28 (DAS-28) formula, and regarding DAS-28, we divided patients into four groups, including remission, low, moderate and high disease activity. There were no significant differences in the plasma level of TNF-α between the newly diagnosed RA patients and subjects who received MTX + HCQ + PSL, as well as healthy controls ($p>0.05$). There was a significant correlation between plasma levels of TNF-α and DAS-28 in the newly diagnosed patients with RA ($r = 0.594$, $P = 0.001$). Targeting TNF-α at the early stage of RA could have more beneficial effects on the amelioration of disease activity.

Keywords: Rheumatoid arthritis. TNF-α. DAS-28. DMARD.

INTRODUCTION

Rheumatoid arthritis (RA) is considered as prevalent chronic inflammatory disorder with a significant social and economic burden (Choy, Panayi, 2001). The clinical presentation of RA is very heterogeneous and consists of the variety of signs and symptoms including joint inflammation and various extra-articular manifestations (Stanich *et al*., 2009). Pro-inflammatory cytokines are among soluble mediators with a crucial role in the pathogenesis of rheumatoid arthritis (Vervoordeldonk, Tak, 2002). TNF-α is one of these cytokines with potent inflammatory effects in the pathogenesis of synovitis (Butler *et al*., 1995). The effectiveness of anti-TNF-α monoclonal antibodies in the treatment of RA highlights its importance in RA pathogenesis (Firestein, 2016). Traditional disease- modifying anti-rheumatic drugs (DMARDs) especially methotrexate (MTX) are the cornerstone of RA treatment (O'Dell *et al*., 1996). The anti-inflammatory effect of DMARDs including MTX, hydroxychloroquine (HCQ), and sulfasalazine (SSZ) could be attributed directly or indirectly to inhibition

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of pro-inflammatory cytokine and chemokines release (Seitz *et al*., 1995, Karres *et al*., 1998, Gronberg, 1994). TNF-α induces production of other pro-inflammatory cytokines such as IL-1 in the inflamed synovium and also acts as a chemoattractant for the different cell types including T lymphocytes (Green *et al*., 1998). TNF-α also promotes the process of bone resorption through its effect on osteoclasts (Lam *et al*., 2000, Abu-Amer *et al*., 1997). Various studies have demonstrated that TNF-α is released from synovium and reaches its systemic levels in blood and exerts its deleterious effects on several tissues (Sattar *et al*., 2003) Although the role of TNF-α as a pro-inflammatory cytokine in the pathogenesis of synovial inflammation has been comprehensively explored, the effect of its plasma level fluctuation on disease activity deserves further investigation (Chu *et al*., 1991, Larsson *et al*., 2015, Matsuno *et al*., 2002). The purpose of our study was to compare the TNF-α plasma levels in the newly established RA and patients who received MTX and HCQ combination plus prednisolone (PSL) therapy. Furthermore we evaluated the correlation between disease activity and plasma concentration of TNF-α in each patients group.

METHODS

Patients characteristics

The study was in accordance with the Declaration of Helsinki and was conducted with approval from the Ethical Committee of Kermanshah University of Medical Sciences (KUMS). Patients were selected by consecutive sampling between July 2017, and October 2017 from Helal Ahmar clinic of Kermanshah University of medical sciences. All participants signed informed consent and were informed about the aim and procedures of this research. All patients were diagnosed according to the EULAR/ACR 2010 classification criteria by an expert rheumatologist. We have included 60 patients with rheumatoid arthritis including 30 newly diagnosed patients (age, 47.3 ± 2.03 years; 6 men and 24 women) without any treatment for RA and 30 patients (age, 47.9 ± 1.8 years; 6 men and 24 women) who received Methotrexate (MTX, 7.5- 25 mg/week), Hydroxychloroquine (HCQ, 200 mg/day) combinational therapy plus oral Prednisolone (PSL, range from 5-10 mg/day).

Patients with the history of other rheumatic and autoimmune disease, severe infection, cancer and pregnant women were excluded from our study. Also, 30 age- and sex-matched healthy subjects (age, 47.6 ± 1.9 years; 6 men and 24 women) were included as the control group in this survey.

Plasma sample collection

5 ml peripheral Blood was obtained from each patient and healthy controls. Plasma samples were separated in (Ethylenediaminetetraacetic Acid) EDTA containing tubes and centrifuged at 3000g for 10 minutes and was stored in -70° centigrade for later measurement of TNF-α.

The calculation of disease activity score

According to the formula, DAS-28 = 0.56 (TJ) ½ +0.28 (SJ) 1/2 +0.70 ln (ESR) +0.014 GH (TJ: Number of tender joints from 28 joints; SJ: Number of swollen joints from28 joints; GH: global health), patients were categorized into four different groups including remission (DAS28 ≤ 2.6), low (2.6 < DAS28 ≤ 3.2), moderate (3.2 < DAS28 ≤ 5.1) and high (DAS28 > 5.1) (Inoue *et al*., 2007).

Method of measuring plasma TNF-α

Plasma level of TNF-α was measured using a sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (IBL kit, Germany).

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS, version 24. The data normality was assessed by the Kolmogorov-*Smirnov* (*K-S*) test. The Kruskal-Wallis Test as a One-way non-parametric ANOVA was used to compare the plasma levels of TNF-α among our groups. The T-test and Mann-Whitney test were used for parametric and non-parametric data respectively. Pearson's correlation test was employed to assess the correlations between parametric variables, also Spearman rank correlations were applied to test the correlation between non-parametric variable. In all statistical analysis ($p < 0.05$) was considered statistically significant and the results are expressed as the mean ± SEM.

RESULTS

Demographic and clinical variables

The demographic and clinical characteristics of patients and controls are shown in Table I. Patients and controls did not significantly differ in age or sex. ESR values and DAS-28 were significantly higher in patients without treatment compared to RA patients who received the combination of MTX and HCQ plus PLS ($p = 0.007$, $p < 0.001$, respectively) (Table I).

The mean plasma levels of TNF-α did not differ significantly between newly diagnosed RA patients, Patients who received the combination of MTX and HCQ plus PSL and also with healthy subjects as shown in Figure 1. (8.039 ± 2.225 pg/mL, 8.326 ± 2.127 pg/mL, 7.824±1.752pg/mL, $P=0.567$, respectively). The increasing trend in mean plasma levels of TNF-α in subgroups of patients with early RA including Remission, low, moderate, and high disease activity have been seen (0.00, 6.7244 ± .37945, 8.7592 ± .63141, 9.5817 ± .64653pg/mL, respectively, $P = 0.007$,) in contrast, there were no significant differences among subgroups in RA patients treated with the combination of MTX and HCQ plus PSL (8.3971 ± .52008, 8.1537 ± 1.17491, 6.8920 ± .24850, 0.00, pg/mL, $P = 0.295$, respectively) (Table II).

The plasma levels of TNF-α had significant correlation with DAS-28 In the newly diagnosed patients ($r = 0.594$, $P = 0.001$) but not in RA patients who were under treatment ($r = - 0.29$, $P = 0.12$). There was no significant correlation between ESR and plasma levels of TNF-α in both newly diagnosed and under treatment groups as shown in Figure 2 ($r = 0.295$, $P = 0.235$; $r = - 0.185$, $P = 0.328$, respectively).

Table I. Demographic and clinical characteristics of rheumatoid arthritis patients and control

Variables		Controls (n=30)	Rheumatoid Arthritis patients	
			Treated (n=30)	Untreated (n=30)
Age	Female (n = 24)	46.13 ± 1.98	46.50 ± 2.07	46.46 ± 2.41
	Male (n = 6)	53.67 ± 4.97	53.50 ± 3.54	50.07 ± 3.21
Min/Max (age)	Female	26/60	26/64	25/67
	Male	40/70	45/67	41/63
		5 ± 0.95		
Drugs (%)	Prednisolone	0	100	0
	Methotrexate	0	100	0
	Hydroxychloroquine	0	100	0
	Other DMARDS	0	0	0
Tests	ESR (mm/h)		15.17 ± 1.79**	28.63 ± 5.26
	Tender joint		1.27 ± 0.24***	6.23 ± 0.66
	Swollen joint		0.73 ± 0.14***	3.07 ± 0.39
	DAS-28		2.43 ± 0.13***	3.96 ± 0.18
	TNF-α (pg/ml)	7.82 ± 1.75	8.04 ± 2.23	8.33 ± 2.13

Data are Mean±SEM; prednisolone dose: 5–10 mg/day, Methotrexatedose: 7.5–25 mg/week, Hydroxychloroquine: 200mg/day*$P < 0.05$,**$P < 0.01$, ***$P < 0.001$. DAS-28, Disease Activity Score-28; DMARD, disease-modifying anti-rheumatic drug; ESR, erythrocyte sedimentation rate; TNF-α, tumor necrosis factor α.

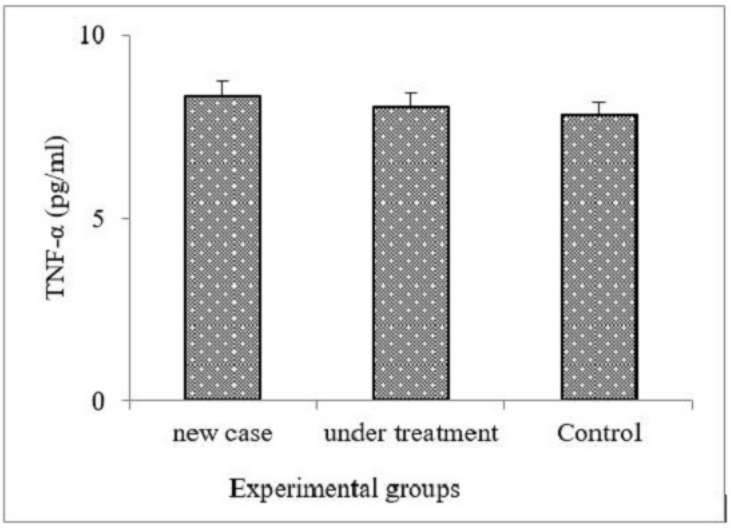

FIGURE 1 - The plasma levels of tumor necrosis factor-α (TNF-α) in the newly diagnosed rheumatoid arthritis (RA) patients, under treatment patients and age and sex-matched healthy subjects.

Table II. - The comparison of plasma levels of TNF-α in new case and under treatment RA patients based on disease activity score- 28

Groups	Disease activity	TNF-α Mean±SEM	Number
New case	Remission =< 2.60		0
	Low = 2.61-3.20	6.72 ± 0.38	9
	Moderate = 3.20-5.10	8.76 ± 0.63*	13
	High => 5.10	9.58 ± 0.65**	8
Under treatment (MTX+HCQ+PSL)	Remission =< 2.60	8.39 ± .52	17
	Low = 2.61-3.20	8.15 ± 1.17	7
	Moderate = 3.20-5.10	6.89 ± 0.25	6
	High => 5.10		0

*p < 0.05 moderate disease activity vs low disease activity in new case patient
**p < 0.001 high disease activity vs low disease activity in new case patient.

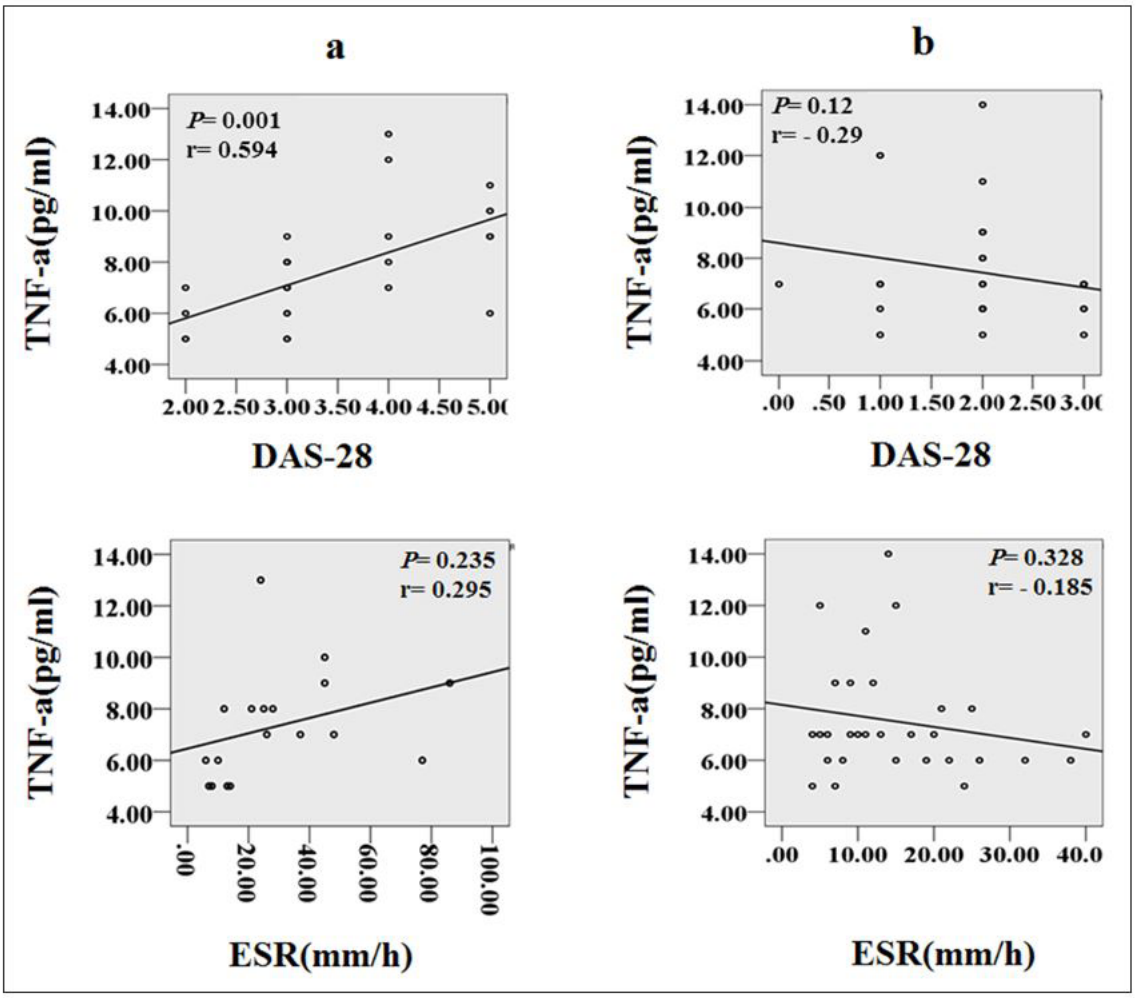

FIGURE 2 - Correlations between study variables. Correlations between TNF-α and DAS-28, TNF-α and ESR, in both new case and under treatment RA patients

DISCUSSION

Although the role of the locally produced TNF-α in the pathogenesis of synovitis and joint damage has been previously determined, the effect of its plasma level on RA disease progression warrants further investigation. (Chu *et al*., 1991; Matsuno *et al*., 2002; Georgopoulos, Plows, Kollias, 1996). In our study, the mean plasma levels of TNF-α was higher in both naive RA patients and patients who received the combination of MTX+HCQ+ PSL in comparison with healthy subjects, but the statistical differences were not significant (P > 0.05).This was contrary to the study of Tetta *et al*. which showed statistically significant differences between the plasma levels of TNF-α in RA patients and healthy subjects (Tetta *et al*., 1990). The probable cause of this controversy is because of the the different method which we used in our study. To measure the plasma levels of TNF-α Tetta *et al*. evaluated the cytotoxic activity of this cytokine while we used high sensitive ELISA kit which directly measured TNF-α with the much lower limit of detection. Similar to our results, Beyazal *et al*. (2017) revealed that the mean serum TNF-α levels are similar between RA patients and healthy subjects (Beyazal *et al*., 2017). In this study, all patients were under treatment with DMARD as monotherapy or in combination whereas in our investigation, we classified patients in two separate groups including newly diagnosed patients and patients who received combinational DMARD including MTX

and HCQ plus prednisolone. Besides that, we could not find significant differences in plasma levels of TNF-α in our newly diagnosed RA compared with patients who were under treatment with MTX+HCQ+PSL. Similar result was observed in the study of Nishina *et al*. (2013) which revealed that monotherapy with MTX does not have any effect on plasma TNF-α levels. As MTX is the cornerstone of combinational DMARD therapy, it can be concluded that TNF-α is not the main target of this regimen. Recently, Nishina *et al*. (2013) demonstrated that following MTX therapy, the plasma levels of IL-6 but not TNF-α will be reduced (Nishina *et al*., 2013). In the following, we evaluated the association between plasma levels of TNF-α and RA disease activity in newly diagnosed RA patients as well as patients with established RA who received therapy. Previous studies showed a positive correlation between plasma levels of TNF-α and disease activity in RA patients, but we just observed this positive correlation in the newly diagnosed patients, not in patients who were undertreatment (Xia *et al*., 2015). In the newly diagnosed patient, the concentration of plasma TNF-α was significantly lower in sub-group with low disease activity compared with the sub-groups with the moderate and high disease activity. It can be concluded that TNF-α has an important role in the early pathologic events of arthritis in naïve RA patients. Our finding is supported by previous data which shows that early aggressive treatment with TNF-α inhibitors and MTX causes durable remission in RA patients (Quinn *et al*., 2005). Moreover animal study demonstrated that the TNF-α is the principal cytokine in the early acute inflammatory process of RA and IL-1β has an important role in the maintenance of ensuing inflammatory reactions (Joosten *et al*., 1999). Interestingly, the previous study revealed that the TNF-α triggers a cascade of inflammatory cytokines release in the synovium of new-onset RA patients, in such a way that neutralization of TNF-α can prevent the production of IL-1 and IL-6, two important cytokines in the pathogenesis of RA (Butler *et al*., 1995).

CONCLUSION

Plasma levels of TNF-α have a significant correlation with disease activity in new-onset RA and it can be concluded that targeting TNF-α in the early stage of RA could have a more beneficial therapeutic outcome.

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DECLRATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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