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Tweak Levels in Rheumatic Inflammatory Diseases

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Abstract; The objective of this study is to investigate and compare serum levels of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) in rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and Behçet's disease. Patients with a diagnosis of RA (n = 20), SLE (n = 20) and Behçet's disease (n = 20) and a healthy control group (n = 19) were included in our study. Disease activity indexes, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were recorded in the patient groups. Serum TWEAK levels were measured with available commercial enzyme linked immunosorbent assay kits. The serum TWEAK levels were significantly higher in all patient groups compared to the control group. However, no significant difference was found in paired comparisons among patient groups. When the patients with high disease activity scores were compared to patients with low disease activity scores in RA, SLE and Behçet's disease subgroups, there was no significant difference in terms of TWEAK levels. Hypertension, atherosclerosis, diabetes mellitus and smoking had no effect on serum TWEAK levels. In correlation analysis, although serum TWEAK levels showed a significant negative correlation with age (r = -0.361, p = 0.005), there was no significant correlation with body mass index (BMI), ESR, and CRP levels. In RA, SLE and Behçet's disease, although different inflammatory pathways and different cytokine release patterns play a role in their pathogenesis, the similar increase in serum TWEAK levels and the absence of a relationship with the disease activity scores reflecting the last stage of inflammation may indicate that the TWEAK / Fn14 pathway plays a role in earlier stages where the inflammatory pathways have not differentiated yet.

INTRODUCTION

The immune system plays a leading role in the pathogenesis of rheumatological diseases. Rheumatologic diseases manifest itself within the framework of a specific inflammatory response, different cytokine patterns and consequently different clinics. Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are autoimmune diseases in which acquired immune response is essential in their pathophysiology. While there is a T-helper-1 type cytokine release pattern in RA, there is a T-helper-2 type cytokine pattern in SLE[1, 2]. On the other hand, Behçet's disease is generally accepted as an autoinflammatory disease because the natural immune response is more prominent in its pathophysiology[3].

Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is a member of the TNF ligand family and is first synthesized as a 249 amino acid transmembrane protein[4]. Although it was initially defined as an apoptosis stimulant[5], it was shown in later studies that it participated in many inflammatory and immunological processes[6, 7]. TWEAK binds to its only known receptor, fibroblast growth factor-inducible 14 (Fn14)[8], and increased TWEAK levels due to inflammation stimulates the release of cytokines such as TNF- α, IL-1, IL-6, granulocyte-colony stimulating factor (G-CSF), and interferon-γ monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 alpha (MIP-1α), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1)[9, 10]. The main source of soluble TWEAK in inflammatory tissue is macrophages / monocytes[11]. These data show that the TWEAK / Fn14 pathway makes significant contributions to inflammation in tissues and indicates that excessive or persistent upregulation of this pathway contributes significantly to the pathogenesis of some inflammatory diseases such as SLE and RA[12-15].

In this study, it was aimed to investigate and compare serum TWEAK levels in RA, SLE and Behçet's disease.

MATERIALS and METHODS

Ethical approval

This study protocol was approved by local ethic committee (OMU-KAEK, approval no. 2020-024).

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Healthy and patient volunteers

Patients with a diagnosis of RA (n = 20), SLE (n = 20), Behçet's disease (n = 20) and a healthy control group (n = 19) who admitted to our rheumatology outpatient clinic between May 1-31, 2020 were included in our study. The study protocol was approved by the local ethics committee. The diagnosis of RA was made according to the 2010 ACR/EULAR RA classification criteria[16], the diagnosis of SLE was made using the 2019 -EULAR/ACR Classification Criteria for Systemic Lupus Erythematosus diagnostic criteria[17] and the diagnosis of Behçet's disease was made according to the International Criteria for Behçet's Disease (ICBD)[18]. In evaluating disease activities, BD current activity form (BDCAF)[19] for Behçet's disease, Disease Activity Score 28-joint (DAS28)[20] for RA and SLE Disease Activity Index 2000 (SLEDAI-2 K)[21] for SLE were used. The gender and age of the patients, medical history, physical examination findings and laboratory data, additional diseases, and smoking status were recorded. The patients with active infection, a diagnosis of malignancy, chronic lung, kidney or liver disease, and heart failure were excluded from the study.

Laboratory analysis

Serums obtained by centrifuging blood samples (Shimadzu UV160A, S.No: 28006648, Japan) at 3000 rpm for 10 minutes stored at -80^{0}C. On the day of analysis, samples were dissolved at room temperature. All analysis was done according to the manufacturer's instructions. Samples showing high concentration were diluted and measured twice.

TWEAK concentrations in serum were measured using the commercially available TWEAK enzyme linked immunosorbent assay (ELISA) (Human Tumour Necrosis Factor Related Weak Inducer of Apoptosis, Cat. No. E1820Hu, Bioassay Technology Laboratory, Shangai, China). Enzymatic reactions were measured in an automatic microplate photometer. TWEAK levels were determined by comparing the optical density of the samples with the standard curve. The mean within-test and within-test percentage coefficients of variation for TWEAK were <10% and <8%, respectively. All experiments were carried out in accordance with the manufacturer's instructions. The expected values of the test were 10-4000 mg / L.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS 11.0, Chicago, IL, USA) was used for the statistical analysis of all data. The results were expressed using the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test were used to determine the statistical differences among the groups. The categorical variables were compared with the chi-square test. The Pearson correlation coefficient was used for correlation analysis. Analysis of covariance (ANCOVA) was also used in order to modify the variables for age, gender, and BMI. Values of $p<0.05$ were considered statistically significant.

RESULTS

The demographic and laboratory data of the patients and healthy volunteers included in the study are summarized in Table 1. The DAS28 was 5.31 ± 1.8 in the RA group, the SLEDAI was 7.8 ± 6.2 in the SLE group, and the BDCAF score was 4.7 ± 2.9 in the Behçet's disease group. There was a significant difference among all groups in terms of age with the ANOVA Test (Table 1). In post-hoc statistical analysis, there was no significant difference in age between the healthy control group and the Behcet's disease group ($p> 0.05$), but there was a significant difference in age in all other paired comparisons among the groups ($p <0.05$).

There was no significant difference between the patient groups in terms of BMI, hypertension, atherosclerosis, diabetes mellitus and smoking, although a significant difference was found in terms of gender (Table 1).

ESR and CRP levels were higher in all patient groups compared to the healthy control group. While there was no difference between the patient groups in terms of CRP level in paired comparisons, the sedimentation level was higher in the RA group than in the Behçet's disease group ($p=0.045$).

Serum TWEAK levels were significantly higher in all patient groups compared to the control group (Figure 1). However, no significant difference was found in paired comparisons among patient groups. When patients with high and low disease activity scores were compared, there was no significant difference in terms of TWEAK levels. Hypertension, atherosclerosis, diabetes mellitus and smoking had no effect on serum TWEAK levels.

Although serum TWEAK levels showed a significant negative correlation with age

($r=-0.361$, $p=0.005$) in correlation analysis, there was no significant correlation with BMI, ESR and CRP levels.

Table 1. Demographics and laboratory data in the study groups.

	Healthy Controls (n:19)	Rheumatoid Arthritis (n:20)	SLE (n:20)	Behcet's Disease (n:20)	P
Age (years)	39.5 ± 10.7	54.9 ± 12.5	48.4 ± 14.2	38.7 ± 10.6	<0.001
Gender (F/M)	10/9	14/6	18/2	10/10	0.022
BMI (kg/m^2)		25.1±5.1	23.4 ±4.9	24.7±5.2	0.923
Smoking	-	5	5	6	0.918
Hypertension	-	6	8	3	0.210
Atherosclerosis	-	2	2	3	0.851
Diabetes Mellitus	-	2	2	2	1.000
ESR (mm/h)	3.38 ± 1.01	46 ± 27.3	35.6 ± 18.3	29.8 ± 17.7	<0.001
CRP (mg/l)	1.42 ± 0.5	19.8 ± 47.1	6.4 ± 7.7	5.6 ± 7.5	0.001
TWEAK (ng/ml)	55 ± 52	1201 ± 872	1187 ± 839	1171 ± 734	<0.001

SLE: Systemic lupus erythematosus, BMI: Body mass index, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, TWEAK: Tumor necrosis factor-like weak inducer of apoptosis

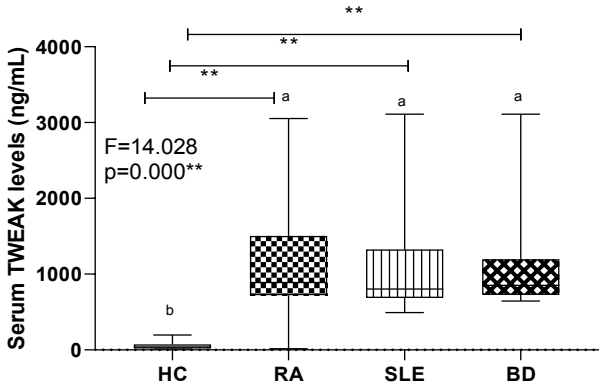

Figure 1. Serum TWEAK levels in the study groups

DISCUSSION

In this study, the serum TWEAK levels of RA, SLE and Behçet's disease patients, in which different immune mechanisms play a role in the etiopathogenesis and consequently present with different clinical findings, were compared with healthy volunteers. Although there are different cytokine release patterns, previous studies have claimed that the TWEAK pathway also plays a role in the pathophysiology of these three diseases. The serum TWEAK levels were significantly higher in patients with RA, SLE, and Behçet's disease when compared with healthy volunteers. However, when these three rheumatic inflammatory diseases were compared with each other, there was no significant difference in terms of serum TWEAK levels. In addition, in the patient groups, no significant difference was found in the serum TWEAK levels of the patients with low and high disease activity. The serum TWEAK levels showed a negative correlation only with the age, but not with the ESR and CRP levels.

El-Shehaby et al.[22] found that urinary TWEAK level was higher in patients with renal involvement compared to those without renal involvement in a case-control study performed in SLE nephritis. On the other hand, Balajkova et al.[23] investigated the role of TWEAK in neuropsychiatric SLE patients and demonstrated that there was no relationship between SLEDAI and serum TWEAK levels, and it was not associated with neuropsychiatric SLE symptoms such as headache, seizure, cerebrovascular disease, cognitive dysfunction and anxiety. Mirioglu et al.[24] determined that serum TWEAK level was increased in both SLE and ANCA-associated vasculitis with active renal involvement and stated that TWEAK was not a specific biomarker for SLE and SLE nephritis. In our study, the increase in the serum TWEAK level in all three patient groups supports the view that TWEAK level is not a specific biomarker for SLE.

There are limited number of studies investigating the role of TWEAK in Behçet's disease. Lopalco et al.[25] showed that serum TWEAK levels were increased compared to healthy controls in their study published in 2017. They also reported that serum TWEAK levels were upregulated both in active and inactive Behçet's disease patients. On the other hand, Icli et al.[26] found that serum TWEAK level was increased in Behçet's disease patients and demonstrated that serum TWEAK level correlated with disease activity. In our study, serum TWEAK levels were increased in Behçet's disease, but were not associated with disease activity similar to the study by Lopalco et al.[25]

In studies conducted to investigate the possible role of the TWEAK / Fn14 pathway in the physiopathology of RA, it was found that TWEAK levels increased in the synovial tissue, synovial fluid and serum of RA patients[27]. In animal models of RA, anti-tweak monoclonal antibodies were observed to provide significant reductions in disease inflammation, joint inflammation, angiogenesis, cartilage, and bone loss[28]. In the Phase I study conducted in RA, it was found that there was a significant decrease in TWEAK levels and inflammatory

markers at the end of the 1st month due to anti-TWEAK monoclonal antibodies[29]. In our study, high TWEAK levels in RA patients compared to the healthy control group indicate that the TWEAK / Fn14 pathway plays a role in the inflammation in RA development.

TWEAK is a pluripotent and multifunctional cytokine that belongs to the TNF superfamily. It has been shown to have active roles in many processes such as cellular proliferation, differentiation, migration, survival, apoptosis, angiogenesis and inflammation[30-32]. Apart from RA, SLE, and Behcet's disease, there are results suggesting that the TWEAK / Fn14 pathway plays a role in diseases such as multiple sclerosis and inflammatory bowel disease[33, 34]. Although there are different cytokine patterns and different inflammatory pathways in the pathogenesis of each of these diseases, they all have high TWEAK levels. In our study, while TWEAK levels were found to be significantly higher in patients with RA, SLE and Behçet's disease, a significant increase in ESR and CRP levels occurred in patients with active disease compared with those in remission whereas there was no significant difference in terms of TWEAK levels. These results suggest that the TWEAK / Fn14 pathway may be involved in a more common and preliminary stage in which inflammatory cascades have not differentiated from each other yet.

The main limitations of this study are its cross-sectional design and small sample size. Failure to find a relationship between TWEAK levels and disease activities in patient groups may be due to the small number of patient groups. Also, investigating for other inflammatory cytokines together would be good to demonstrate their association with different cytokine release patterns. Moreover, we believe that it is also important to measure and compare TWEAK levels in these rheumatic inflammatory diseases known to have different cytokine release patterns.

In conclusion, the increase in serum TWEAK levels in RA, SLE and Behçet's disease, which are rheumatic inflammatory diseases, suggests that it plays a role in the pathophysiology of these diseases. The similar increase in TWEAK levels in these diseases of different nature and the absence of a relationship with CRP and disease activity indexes, which mostly suggests the last stages of inflammation, indicate that the TWEAK/Fn14 pathway plays a role in earlier stages where the inflammatory pathways have not differentiated yet.

Conflict of interest

The authors declare that they have no conflict of interest

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