

Assessment of serum TWEAK levels in patients with familial Mediterranean fever

Gökhan Yavuzbilge¹, Muhammed Okuyucu², Yeşim Civil³, Serkan Günaydın¹, Bahattin Avcı³

¹ Department of Rheumatology, Medical Faculty, Ondokuz Mayıs University, Samsun, Turkey

² Department of Internal Medicine, Medical Faculty, Ondokuz Mayıs University, Samsun, Turkey

³ Department of Biochemistry, Medical Faculty, Ondokuz Mayıs University, Samsun, Turkey

ORCID ID of the author(s)

GY: 0000-0002-8879-8859
MO: 0000-0002-6026-2024
YC: 0000-0002-9662-244X
SG: 0000-0002-1131-2531
BA: 0000-0001-6471-6495

Corresponding Author

Gokhan Yavuzbilge
Ondokuz Mayıs University, Medical Faculty,
Department of Rheumatology, Korfez Mahallesi,
Ondokuz Mayıs University, 55270, Atakum,
Samsun, Turkey
E-mail: gokhany_89@hotmail.com

Ethics Committee Approval

The study protocol was approved by Ondokuz Mayıs University ethics committee (OMU-KAEK, 1.11.2020, approval no. 2020/669). All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest

No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Mediterranean fever is an autoinflammatory disease characterized by recurrent attacks. Tumor necrosis factor (TNF) - like weak inducer of apoptosis (TWEAK) is a member of the TNF ligand family, and it has been reported to contribute significantly to the initiation of many inflammatory and immunological processes. In previous studies, an increasing amount of evidence has implicated the participation of TWEAK / Fn14 pathway in the pathogenesis of rheumatic inflammatory diseases that include rheumatoid arthritis, systemic lupus erythematosus and Behçet's disease. The aim of this study was to investigate the serum TWEAK levels of patients with Familial Mediterranean fever (FMF) and its possible relationship with inflammatory markers and disease activity.

Methods: Our study included 20 patients with FMF and 19 healthy volunteers. Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) were measured, and PRAS disease severity score was determined in patients with FMF. Also, the FMF attack period was questioned. Serum TWEAK levels were measured with available commercial Enzyme Linked Immunosorbent Assay kits.

Results: There was no significant difference in terms of age and gender between the FMF group and the healthy control group ($P>0.05$, for all). ESR, CRP and serum TWEAK levels were significantly higher in patients with FMF ($P<0.001$ for all). PRAS score in FMF patients was 3.4. Serum TWEAK level was not correlated with ESR ($r=-0.042$, $P=0.0801$), CRP ($r=-0.017$, $P=0.921$), or PRAS score ($r=0.247$, $P=0.149$). The ESR and CRP levels of patients in FMF attack period were significantly higher compared to attack free period ($P<0.001$ for both) whereas there was no significant difference in serum TWEAK levels ($P=0.686$).

Conclusions: Serum TWEAK levels are increased in FMF disease with attacks. However, this increase is not associated with increased ESR and CRP during FMF attacks. These results indicate that the TWEAK / Fn14 pathway plays a role in earlier stages where the inflammatory pathways have not differentiated yet. Serum TWEAK levels appear to be more successful in reflecting a lower degree of inflammation compared to ESR and CRP.

Keywords: TWEAK, Familial Mediterranean fever, Inflammation, Biomarker

Introduction

Familial Mediterranean fever (FMF) is the most common autoinflammatory disorder in the world [1, 2]. It is an autosomal recessive disease that is caused by the MEFV (MEDiterranean FeVer) gene and manifests itself as recurrent attacks of fever and short-lived inflammation in the serosal membranes, joints and skin [1, 3]. It is more common among Turks, Arabs, Sephardic Jews and Armenians living in the Mediterranean region [1]. Like other autoinflammatory diseases, it is characterized by abnormalities in the innate immune system.

Before the discovery of the MEFV gene, it was commonly known that FMF attacks were caused by an increase in polymorphonuclear leukocytes in serosal membranes, joints and some areas of the skin. After the discovery of the MEFV gene, research focused on the potential role of pyrin protein in FMF. Pyrin, released from neutrophils and monocytes, has a significant role in the initiation of inflammation and the activation of potent pyrogenic cytokine interleukin (IL) -1 β [4, 5]. It is involved in the activation of caspase-1 which is a structural part of the inflammasome complex and the release of active IL-1. Pyrin activity occurs at the level of the cytoskeletal assembly [6, 7]. In FMF patients, specific microtubule assembly inhibitors prevent pyrin-mediated caspase-1 activation and secretion of IL-1 in peripheral blood mononuclear cells.

Tumor necrosis factor (TNF) - like weak inducer of apoptosis (TWEAK) is a member of the TNF ligand family and first synthesized as a 249 amino acid transmembrane protein [8]. Although it was initially defined as an apoptosis stimulant [9], it was shown in later studies that it is involved in many inflammatory and immunological processes [10, 11]. TWEAK binds to its only known receptor, fibroblast growth factor-inducible 14 (Fn14), and increased TWEAK levels due to inflammation stimulate 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-1 α), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) [12-14]. The main source of soluble TWEAK in inflammatory tissue is macrophages / monocytes [15]. 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 rheumatic inflammatory and infective diseases [16-20].

Behçet's disease is also considered an autoinflammatory disease and common in our country [21]. The fact that anti-TNF treatments are useful in the treatment of Behçet's disease and the results of a previous study suggest that the TWEAK pathway also plays a role in the pathogenesis of Behçet's disease [22]. From this point of view, in this study, we aimed to investigate the serum TWEAK levels and its possible relationship with disease activity in FMF.

Materials and methods

Healthy and patient volunteers

Patients with FMF (n=40) and healthy volunteers (n=38) who visited our rheumatology outpatient clinic between

May 1-31, 2020 were included in our study. The study protocol was approved by the local ethics committee. FMF diagnosis was made on the basis of the Tel-Hashomer or Livneh diagnostic criteria [23]. The disease severity score was determined with the PRAS score in FMF patients. Gender, age, anamnesis, physical examination findings, laboratory data, comorbidities, and smoking history were recorded for all participants. Individuals 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 were stored at -80°C. On the day of analysis, samples were dissolved at room temperature. All analysis was performed 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 Enzyme Linked Immunosorbent Assay (ELISA) kit (Human Tumour Necrosis Factor Related Weak Inducer of Apoptosis, Cat. No. E1820Hu, Bioassay Technology Laboratory, Shanghai, 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. When determining serum TWEAK levels, all ELISA kit studies were carried out in accordance with the manufacturer's instructions. The expected values of the test were 10-4000 mg / L.

Statistical analysis

A sample size of 35 persons per group was calculated based on a power of 85% and a P value of 0.05 (G*power 3.1). 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 with 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 FMF patients and healthy volunteers are summarized in Table 1.

Table 1: Demographics and laboratory data in the study groups

	Healthy Controls (n:38)	FMF (n:40)	P-value
Age (years)	32.5 (10.71)	31.85 (10.64)	0.857
Gender (F/M)	21/17	20/20	0.762
BMI (kg/m ²)	23.5 (3.4)	24.7 (5.2)	0.072
ESR (mm/h)	3.38 (1.01)	29.8 (17.7)	<0.001
CRP (mg/l)	3.5 (0.8)	5.6 (7.5)	<0.001
TWEAK (ng/ml)	55 (52)	1313 (756)	<0.001

FMF: Familial Mediterranean fever BMI: Body mass index, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, TWEAK: Tumor necrosis factor-like weak inducer of apoptosis

There was no significant difference between the two groups in terms of age and gender. ESR, CRP and serum

TWEAK levels were significantly higher in the FMF patients ($P<0.001$).

It was determined that gender, obesity, hypertension, atherosclerosis, diabetes mellitus and smoking had no effect on serum TWEAK levels in FMF group (for all $P>0.05$).

PRAS score in FMF patients was 7.6 (2.3). There was no correlation between age ($r=-0.128$, $P=0.430$), ESR ($r=-0.042$, $P=0.0801$), CRP ($r=-0.017$, $P=0.921$), PRAS ($r=0.247$, $P=0.149$) score and serum TWEAK levels. The ESR (59.9 (25.6) & 24.9 (15.6), $P<0.001$) and CRP (47.2 (23.1) & 2.6 (2.6), $P<0.001$) levels of patients in FMF attack period were significantly higher compared to the attack free period while there was no significant difference in serum TWEAK levels (1303 (817) & 1351 (845) ng/ml, $P=0.686$).

Discussion

In this study, it was investigated whether the TWEAK/Fn 14 pathway was also activated in FMF disease since it had a significant role in the etiopathogenesis of some rheumatic inflammatory diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and Behçet's disease. Serum TWEAK levels were significantly higher in FMF patients compared to healthy volunteers. However, serum TWEAK levels were not associated with ESR, CRP and disease severity score in FMF patients. Moreover, serum TWEAK levels were similar during the attack and attack-free period.

In a study conducted to investigate the possible role of the TWEAK / Fn14 pathway in the pathogenesis of RA, it was found that the expression and serum levels of TWEAK increased in the synovial tissue, synovial fluid and serum of patients with RA [24]. In an experimental model of RA, anti-tweak monoclonal antibodies were observed to provide significant reductions in disease inflammation, joint inflammation, angiogenesis, cartilage and bone loss [25]. There was significant decrease in serum TWEAK levels and inflammatory markers at the end of the 1st month due to anti-TWEAK monoclonal antibodies in a phase 1 study conducted in RA [26]. To best of our knowledge, our study is the first investigating the serum TWEAK levels in FMF patients. The TWEAK / Fn14 pathway, which has been shown to make significant contributions to the pathogenesis of an inflammatory disease such as RA, appears to be activated in FMF.

TWEAK is a pluripotent and multifunctional cytokine that belongs to the TNF superfamily. In previous studies, it has been revealed the TWEAK / Fn14 pathway has an effective role in many different processes such as inflammation, chemotaxis, proliferation of inflammatory cells, differentiation, apoptosis and angiogenesis [27-29].

It is interesting that the TWEAK / Fn14 pathway is activated in many diseases with different etiopathogenesis such as SLE, Behçet's disease, inflammatory bowel disease, and multiple sclerosis [30, 31]. In our study, the high serum TWEAK levels in FMF patients compared with the healthy control group suggests that the TWEAK / Fn14 pathway is also activated in FMF. However, there was no correlation between ESR, CRP and serum TWEAK levels and there was no significant difference in serum TWEAK levels of patients in FMF attack period comparing to that obtained during attack-free period. The fact

that the TWEAK / Fn14 pathway is activated in diseases with different cytokine release patterns and does not show a significant correlation with disease activity in FMF suggests that the TWEAK / Fn14 pathway can be involved in a more common and preliminary stage in which inflammatory cascades have not differentiated yet. Another possibility is the presence of minimal inflammation in FMF even during the attack-free period. According to the results of the study conducted by Kehribar et al. [20], the detection of high serum TWEAK levels despite normal ESR and CRP values in patients with asymptomatic COVID-19 infection supports our view.

Limitations

The main limitations of this study are its cross-sectional design and small sample size. The results might be different if we had more patients for comparing the serum TWEAK levels during the attack and attack-free periods. In addition, if the control group consisted of patients with an inflammatory disease instead of healthy volunteers, some other inflammatory cytokines could be evaluated and their relationship with different cytokine release patterns could be demonstrated. However, we think that our study is important since it is the first study investigating serum TWEAK levels in FMF.

Conclusions

In conclusion, serum TWEAK levels are increased in FMF disease with attacks. However, this increase is not associated with increased ESR and CRP during FMF attacks. Although different cytokine release patterns are demonstrated in previous studies, it has also been shown that the TWEAK / Fn14 pathway is activated in many diseases. When the previous studies and the results of this study are combined, it can be suggested that the TWEAK / Fn14 pathway plays a role in the earlier stages where the inflammatory cascades are not differentiated yet. Serum TWEAK levels appear to be more successful in reflecting a lower degree of inflammation compared to ESR and CRP.

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