Evaluatıon of Dynamic Thiol/Disulfide Homeostasis in Patients with Familial Mediterranean Fever

Ailesel Akdeniz Ateşi Olan Hastalarda Dinamik Tiyol / Disülfid Homeostazının Değerlendirilmesi

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Abstract

Objective: Familial Mediterranean fever (FMF) is a hereditary autoinflammatory disease that includes recurrent episodes of serosal inflammation, with accompanying fever. Thiol/disulfide homeostasis, marker of oxidative stress, is associated with an increase in inflammatory cytokines in many inflammatory diseases. The aim was to evaluate the dynamic thiol/disulfide homeostasis in patients with FMF during attack free period.

Material-Method: In our study, 60 patients with FMF during attack free period, 60 patients with Ankylosing Spondylitis (AS) during inactive period and 60 age-and sex matched controls were included. Native thiol, total thiol and disulfide levels were measured by using the Erel and Neselioglu method.

Results: Total thiol and disulfide levels were found to be significantly lower in FMF group compared to healthy controls. Native thiol levels were detected to be significantly higher in FMF group compared to AS group. However, no significant difference was observed in total thiol levels between FMF and AS group. Ratios obtained using plasma native thiol, total thiol, and disulfide levels differed significantly between the FMF, AS and the control groups.

Conclusions: The lower thiol level of FMF group compared to AS and controls suggests that the thiol / disulfide balance is affected differently in diseases with different pathophysiology. Although the disease is in clinically inactive period, inflammation may continue at different levels in different rheumatic diseases.

Keywords: Ankylosing Spondylitis, Familial Mediterranean Fever, Thiol/disulfide

Özet

Amaç: Ailesel Akdeniz Ateşi (AAA), ateş ile birlikte tekrarlayan serozal inflamasyon ataklarını içeren, kalıtsal bir otoinflamatuar hastalıktır. Oksidatif stresin belirteci Tiyol / disülfid homeostazı, birçok inflamatuar hastalıkta inflamatuar sitokinlerde artış ile ilişkilidir. Çalışmamızın amacı atak dışı dönemde AAA olan hastalarda dinamik tiyol / disülfid homeostazisini değerlendirmektir.

Materyal-Metot: Çalışmamıza atak dışı dönemde 60 AAA'li, 60 inaktif dönemde Ankilozan Spondilit (AS)'li hasta ve 60 yaş ve cinsiyet olarak benzer kontrol grubu dahil edildi. Erel ve Neselioğlu yöntemi kullanılarak nativ tiyol, total tiyol ve disülfid düzeyleri ölçüldü.

Bulgular: AAA grubunda total tiyol ve disülfid düzeyleri sağlıklı kontrollere göre anlamlı derecede düşük bulundu. AAA grubunda nativ tiyol düzeylerinin AS grubuna göre anlamlı olarak yüksek olduğu saptandı. Bununla birlikte, AAA ve AS grubu arasında total tiyol düzeylerinde anlamlı bir farklılık izlenmedi. Plazma nativ tiyol, total tiyol ve disülfid seviyeleri kullanılarak elde edilen oranlar, AAA, AS ve kontrol grupları arasında önemli ölçüde farklıydı.

Sonuç: AAA hastalarında AS ve control grubuna göre düşük tiyol düzeyi saptanması, farklı patofizyolojiye sahip hastalıklarda tiyol / disülfid dengesinin farklı şekilde etkilendiğini düşündürmektedir. Hastalık klinik olarak inaktif bir dönemde olmasına rağmen, farklı romatizmal hastalıklarda inflamasyon farklı düzeylerde devam edebilir.

Anahtar kelimeler: Ankilozan Spondilit, Ailesel Akdeniz Ateşi, Tiyol / Disülfid

Introduction

Familial Mediterranean Fever (FMF) is an autosomal recessive auto-inflammatory disease with recurrent attacks of serositis and fever. It is closely related to ethnic origin. It is more common in Turks, Arabs, Armenians and Jews. Most of the patients have their first episode between 5 and 15 years. Family history is present in 30-50% of patients. FMF occurs as a result of point mutations in the MEFV gene located on the

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short arm of chromosome 16 encoding pyrin protein. Eighty five percent of patients have genetic mutations in exon 2 and exon 10 (1). Colchicine is the main treatment for controlling acute attacks, reducing chronic and subclinical inflammation and prevention of complications. Colchicine is a safe medication that can be well tolerated.The most common FMF complication is amyloidosis. Nonspecific acute phase responses such as erythrocyte sedimentation rate (ESR), fibrinogen, C-reactive protein (CRP) and serum amyloid A (SAA) level are found high during the attack period. IL-1b is an important cytokine in the pathogenesis of FMF (2, 3, 4). In the attack-free period, IL-1b is detected at normal or low levels. Despite the use of colchicine, CRP-SAA levels can be found high in patients without attack period. In addition, inflammatory markers such as lipoprotein a, homocysteine, adrenomedulin, IL-6-8-10, tumor necrosis factor (TNF), interferon-y have been found to be elevated in attack-free period. Continuation of subclinical inflammation in attack-free period suggests that oxidative balance is impaired and oxidative tissue damage continues (5, 6, 7).

Thiol is an important antioxidant molecule that plays a role in the cleansing of reactive oxygen molecules. Primary targets of reactive oxygen molecules are thiol groups of sulfur-containing amino acids in proteins. The plasma thiol pool is consisted of mainly albumin and other proteins (8). Thiol groups in the environment are oxidized by reactive oxygen molecules and reversible disulfide bonds are formed. This conversion is the earliest finding of oxygen radical mediated protein oxidation. The dynamic thiol / disulfide balance has critical functions such as antioxidant defense, detoxification, apoptosis, regulation of enzyme activities, cellular signal transduction (9). Abnormal thiol / disulfide balance is involved in the pathogenesis of many inflammatory and non-inflammatory diseases such as diabetes mellitus, malignancy, cardiovascular diseases, chronic renal failure, rheumatoid arthritis (10, 11, 12). The fact that the thiol / disulfide balance is measured on both sides by a practical, uncomplicated and inexpensive method described by Erel and Neselioglu has increased the interest to this area (13). In this study, we aimed to investigate the dynamic thiol / disulfide balance in attack-free period in FMF patients.

Material and Methods

Sixty patients who had FMF diagnosis according to the Tel-Hashomer criteria (1)] and 60 age and sex matched controls were included in the study. FMF patients were enrolled to the study during attack-free period. All FMF patients were receiving colchicine treatment. A total of 60 patients who have been diagnosed with ankylosing spondylitis (AS) according to Modified New York classification criteria (15) and whose score in The Bath ankylosing spondylitis Disease Activity Index (BASDAI) (16) were below 4 (inactive) were included in the study as the control group. FMF and AS patients were assessed by the same rheumatologist, with physical and laboratory examinations. The patients who had chronic diseases (hypertension, diabetes mellitus, hyperlipidemia, obesity, autoimmune diseases, kidney disease, etc.) and recent history of infection, smoking and alcohol use and who had taken immunosuppressive therapy (in the last 4 weeks before taking blood sample for the study) were excluded from the study.

Blood samples were taken from the patients included in the study after an 8 hour fasting. Fasting blood glucose (FBG), alanine aminotransferase (ALT), creatinine, whole blood count, CRP, ESR were measured in all patients. ESR was calculated with Alifax THL1 ESR analyzer (Alifax SPA, Padua, Italy) and creatinine and ALT were assessed with Beckman AU 5800 Autoanalyzer (Beckman Coulter Inc., USA). For finding thiol / disulfide ratio, 2 ml of venous blood was taken into the tubes containing ethylenediaminetetraacetic acid (EDTA), and the plasma portion was separated by centrifugation for 10 minutes. The samples were stored at -80°C until the day of analysis. Native thiol (NT), total thiol (TT) and disulfide levels were assessed by Erel&Neselioglu automated measurement method (13).

Local ethics committee approval for the study was obtained. All patients and controls have signed an informed consent form stating that they volunteered to participate in the study.

Biochemical Analysis

NT, TT and disulfide levels were measured by the spectrophotometric method developed by Erel&Neselioglu (13). Dynamic disulfide bonds were separated from functional thiol groups using sodium borohydride (NaBH4). Unused NaBH4 in the medium was removed by the addition of formaldehyde. By this way, the formation of disulfide bonds after the use of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) is reduced. TT level was calculated by Modified Ellman method. The half of the difference between serum TT and NT were defined as disulfide level. Disulfide / NT, disulfide / TT, NT / TT ratios were calculated by using these data.

Statistical analysis:

The data were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corp., Armonk, New York, USA). Descriptive statistics were determined as frequencies, percentages, mean, and standard deviation (SD). Normally distributed continuous variables were given as mean ± standard deviation, non-normal distribution variables were given as median (interquartile range). Nonparametric Kolmogorov-Smirnov test was used for evaluating normally distributed data, and non-normally distributed data were assessed by using the Mann-Whitney U test. Correlation analysis was performed by Pearson correlation test. P values less than 0.05 were considered statistically significant.

Results

The mean age in our study was 28.2±9.6 years in the FMF group, 31.8±6.9 years in the AS group and 30.2±7.2 years in the control group. In all groups, 50% of the patients were female. Body mass index (BMI) in the FMF group was 23.7±1.8kg/m2, in the AS group was 25.4±4.3 kg/m2 and in the control group was 26.1±3.8 kg/m2. No statistically significant difference was found between patient and control groups in terms of clinical and laboratory data. Clinical and laboratory findings of the patient and control groups were given in Table 1.

Table 1. Demographic and laboratory data of patients' and controls

Parameter	FMF (n=60)	AS (n=60)	Controls (n=60)
Age, years	28.2±9.6	31.8±6.9	30.2±7.2
Sex (F/M), n	30/30	30/30	30/30
BMI, kg/m2	23.7±1.8	25.4±4.3	26.1±3.8
FBG, mg/dL	91.3±8.4	88.0±4.9	93.0±7.6
Creatinine, mg/dL	0.85±0.3	0.74±0.26	0.89±0.2
ALT, U/L	17±12.8	26.4±9.7	20.6±7.4
ESR, mm/h	16.4±6.9	18.1±7.4	11.9±4.9
CRP, mg/L	1.8±0.6	1.6±0.5	2.4±1.1
TSH,	2.7±1.8	2.4±1.1	1.7±1.0
Hemoglobin, g/dL	12.9±1.07	14±2.4	13.7±2.9

Abbreviations: FMF: Familial Mediterranean Fever, AS: Ankylosing Spondylitis, BMI: Body mass index, FBG: Fasting blood glucose, ALT:Alanine aminotransferase, ESR: Erytrocyte Sedimentation Rate, CRP: C-reactive protein, TSH: Thyroid stimulating hormone
Values are presented as mean ± standard deviation

Table 2. Plasma thiol - disulfide levels of FMF and control group

Parameter	FMF (n=60)	Controls (n=60)	p
Native thiol, µmol/L	330.79±56.05	339.17±38.3	0.34
Total thiol, µmol/L	353.55±55.5	372.64±40.8	0.03*
Disulfide, µmol/L	11.3±6.2	16.7±6.5	0.001*
Disulfide/Native thiol, %	3.6±2.4	5.0±2.1	0.001*
Disulfide/Total thiol, %	3.2±2.0	4.4±1.7	0.001*
Native thiol/Total thiol, %	93.4±4.0	91.01±3.4	0.001*

Abbreviations: FMF: Familial Mediterranean Fever
Values are presented as mean ± standard deviation
* P < 0,05 is considered statistically significant

Table 3. Plasma thiol - disulfide levels of FMF and AS

Parameter	FMF (n=60)	AS (n=60)	p
Native thiol, µmol/L	330.79±56.05	307.39±41.0	0.01*
Total thiol, µmol/L	353.55±55.5	336.85±45.4	0.07
Disulfide, µmol/L	11.3±6.2	14.7±7.0	0.007*
Disulfide/Native thiol, %	3.6±2.4	4.8±2.3	0.007*
Disulfide/Total thiol, %	3.2±2.0	4.3±1.9	0.004*
Native thiol/Total thiol, %	93.4±4.0	91.3±3.8	0.004*

Abbreviations: FMF: Familial Mediterranean Fever, AS: Ankylosing Spondylitis,
Values are presented as mean ± standard deviation
* P < 0,05 is considered statistically significant.

There was no statistical difference in FMF and control group at NT level (p=0.34). TT was measured as 353.55±55.5 µmol / L in the FMF group and 372.64±40.8 µmol / L in the control group. The decrease in the TT level detected in the FMF group was statistically significant (p=0.03). Disulphide, disulphide / NT, disulphide / TT were significantly lower in FMF patients than in the control group (Table 2). When the FMF and AS groups were compared, the NT and TT levels were lower in the AS group. The decrease in TT level was not statistically significant. Disulphide, disulphide / NT, disulphide / TT ratios were significantly higher in AS group compared to FMF group (Table 3).
According to correlation analysis, there was no statistically significant correlation between thiol / disulphide results and clinical and laboratory parameters.

Discussion

FMF is an autosomal recessive disease with recurrent episodes of serositis and fever andhas no clinical manifestation in attack-free periods. Th1 polarization plays a key role in FMF pathogenesis. Although no clinical findings were detected in the attack-free period, studies in this period have found that acute phase reactants such as SAA, IL-6-8, TNF, interferon-y are higher and there is over-activation of Th1 in peripheral blood (17,18,19). The low level of IL-10, an important T cell inhibitor, suggests that T cell activity cannot be suppressed effectively in attack-free period (20). Continuation of subclinical inflammation in attack-free period suggests that oxidative balance is impaired and oxidative tissue damage continues. In our study, the level of thiol, an important antioxidant, was found to be statistically insignificantly lower than controls in FMF patients in attack-free period. This suggests that although the disease is clinically inactive, cellular damage due to impaired oxidant / antioxidant balance is maintained. In addition, it was determined that the thiol level was lower in the inactive AS patient group when compared with FMF group. Although the level of disulfide, an oxidant molecule, was expected to be high in the disease, in our study, it was seen that it was lower in AS and FMF patients than controls. This suggests that the oxidant / antioxidant balance is in favor of antioxidant reduction in inactive periods of two rheumatic diseases.
Thiol is an important antioxidant molecule that contains sulfhydryl groups and protects the organism from oxidative damage. Thiol reacts with oxidant molecules to form reversible disulfide bonds. This balance between thiol / disulfide is important for many vital activities such as detoxification, regulation of enzymatic reactions and apoptosis. This bilateral equilibrium can be measured by a new method developed by Erel&Neselioglu and it is seen that this equilibrium is defective in many inflammatory diseases such as FMF, juvenile arthritis, celiac disease and non-inflammatory diseases such as subclinical hypothyroidism, sleep apnea syndrome, schizophrenia, hypertension (21, 22, 23, 24, 25). In a study conducted by Yücel et al. with FMF-diagnosed pregnant subjects, lower levels of TT and NT and higher disulfide levels were found in FMF patients when compared to controls. The results were found to be

more advanced in patients with ante-natal complications (26). In another study of FMF thiol / disulfide balance, it was found that FMF patients had lower levels of NT and TT than controls. In this study, disulfide levels were found to be lower compared to controls, as in our study (27).

In the study of Omma et al., TT and NT levels were found to be lower in FMF patients compared to controls When FMF patients were grouped according to whether they were in attack or attack-free period, there was a further decrease in thiol levels during the episode. The disulfide levels were higher in the FMF group, especially in patients with attack, but not statistically significant (28). In our study, the thiol level in the FMF group was found to be low in the attack-free period, similar to these studies. When two different pathophysiologic diseases such as AS and FMF were compared, thiol suppression was found to be more frequent in the AS group. This may suggest that subclinical inflammation continues in inactive periods of both diseases and differs between diseases.Disulfide levels and disulfide ratios were measured in patients as being lower than controls. Contrary to expectations, there may be a number of reasons for disulfide levels to be low in both diseases. Decreased oxidant molecules may be seen due to medical treatment. Another reason is that oxidant molecules may not be actively involved in diseases.In our previous study (29), we found that thiol / disulfide balance was impaired in active and inactive periods of AS patients, but in this study we found that thiol / disulfide balance was altered during inactive periods of diseases with different pathophysiology.

Our study has some limitations. First of all, the study included limited number of patients and it had a cross-sectional design. Another limitation is that patients continued to be treated with colchicine and the oxidant / antioxidant balance may be affected by this treatment.Despite these limitations, enrolling patients with another disease that has different pathophysiological features in inactive period has increased the power of the study. Another powerful aspect of this study is the elimination of co-morbid diseases and conditions that would affect the oxidant / antioxidant balance.

Conclusion

Although FMF is an episodic disease, it has been observed in our study that there is an oxidant / antioxidant imbalance in the attack-free period. This may be due to continued subclinical inflammation in the attack-free period. The lower thiol level of FMF group compared to AS and controls suggests that the thiol / disulfide balance is affected differently in different pathophysiologic diseases. Although the disease is in clinically inactive period, inflammation may continue at different levels in different rheumatic diseases. The fact that thiol / disulfide balance is measured by the Erel and Neselioglu method in a more practical, quick and inexpensive way and increased knowledge about thiol / disulfide balance strengthen the connection between clinical and molecular sciences.

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Conflict of Interests

The authors declare that there are no conflicts of interest.

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