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CLINICAL STUDY

Cu/Zn-superoxide dismutase, paraoxonase and arylesterase activities and malondialdehyde levels in patients with familial mediterranean fever

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Abstract: *Objectives:* In this study, alterations in antioxidant enzyme activities and malondialdehyde (MDA) levels in the serum samples of patients with familial Mediterranean fever (FMF), an autosomal recessive disease characterized by recurrent episodes of peritonitis, pleuritis, arthritis and fever, were investigated and compared with those of age- and sex-matched healthy controls.
Methods: Twenty-five patients with FMF undergoing colchicine therapy at doses of 1–1.5 mg and 25 age- and sex-matched healthy controls were included in the study. In the patients with FMF and control subjects, the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) level were measured. Cu/Zn-superoxide dismutase (Cu/Zn-SOD), paraoxonase-1 (PON-1) and arylesterase (ARE) enzyme activities and MDA levels as a production of lipid peroxidation were evaluated using the appropriate methods.
Results: No statistically significant differences in the serum levels of ESR, CRP, Cu/Zn-SOD, MDA and PON-1 between the groups were observed ($p > 0.05$). Serum ARE activity was significantly decreased in the patients with FMF compared with the control subjects ($p < 0.01$).
Conclusion: In conclusion, some abnormalities in the antioxidant defense system and lipid peroxidation may be observed in FMF patients during attack-free periods. However, further long-term studies on the subject are needed to explore altered lipid peroxidation and antioxidant defense mechanisms in patients with FMF *(Tab. 1, Fig. 1, Ref. 35)*. Full Text in PDF *www.elis.sk.*
Key words*:* familial Mediterranean fever, antioxidants, Cu/Zn-superoxide dismutase, paraoxonase, arylesterase, malondialdehyde.

Familial Mediterranean fever (FMF) is an inherited disorder characterized by recurrent bouts of fever and peritonitis, pleuritis, skin lesions, arthritis and pericarditis; FMF is a chronic relapsing inflammatory process (1, 2). The etiology and pathogenesis of FMF is a multifactorial process. Oxidative stress is thought to play an important role in the pathogenesis of a number of disorders, such as diabetes mellitus, panic disorder and inflammatory bowel diseases (3, 4). Oxidative damage occurs in cells when the critical balance between the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is upset (5).

Free radicals, highly reactive entities and very short-lived molecules, are constantly produced in a wide variety of normal physiological functions. Recently, compelling evidence has demonstrated that oxidative damage is involved in the pathogenesis of inflammatory disorders. Increased ROS and other free radicals, which play an important role in the inflammatory process and contribute to tissue destruction, can initiate lipid peroxidation and DNA damage. This can lead to mutagenesis, carcinogenesis and cell death (5, 6). Decreased enzyme activity in the antioxidant system and increased levels of free radicals in patients with FMF may also have a role in tissue damage.

Antioxidants are the first line of defense against free radical damage. Antioxidant enzymes, such as Cu/Zn-superoxide dismutase (Cu/Zn-SOD), paraoxonase-1 (PON-1) and arylesterase (ARE), play an important role in the protection of cells and tissues against free radical tissue damage. Cu/Zn-SOD acts to eliminate superoxide anion radicals and hydrogen peroxide. In a variety of studies, Cu/Zn-SOD has been shown to protect the tissues from the harmful effects of inflammation (7).

Paraoxonase is a calcium-dependent ester hydrolyses which has both PON-1 and ARE activities. In addition, the enzyme's activity is inversely related to oxidative stress (8, 9). Therefore, PON-1 has been considered as an anti-oxidant enzyme (10). PON-1, which is known to catalyze the hydrolysis of organophosphates and is a 45-kDa glycoprotein, is recognized as an antioxidant enzyme as it hydrolyzes lipid peroxides (11). ARE of human serum is also a designated aromatic esterase, and its activity has most often been measured with phenylacetate as the substrate. ARE, like PON-1, requires calcium for its activity, and is inhibited by

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EDTA (12). Serum PON-1 and ARE combination have been demonstrated to function as a single enzyme. PON-1 and ARE activities are closely related to oxidation and the inflammatory process (13). Malondialdehyde (MDA) is an indicator of oxidative stress and one of the final decomposition products of lipid peroxidation, which has numerous deleterious effects on biological systems. Researchers have reported that the deficient function of antioxidant systems activities and increased ROS production may stimulate the formation of MDA and play a role in the pathogenesis of many diseases, including FMF (14, 15).

We could not find any study in literature that assessed the serum Cu/Zn-SOD, PON-1, ARE and MDA levels in patients with FMF. Therefore, in this study, we evaluated antioxidant enzyme activities and MDA levels in the serum samples of patients with FMF during attack-free periods. The data obtained were compared to those from healthy control subjects.

Material and method

Twenty-five adult patients who fulfilled the clinical diagnostic criteria for FMF were consecutively included in the study. All participants were informed on the study protocol, and their written informed consent was obtained according to the Declaration of Helsinki. The diagnosis of FMF was established according to previously described criteria (16). All patients were undergoing colchicine therapy at doses of 1 – 1.5 mg/day. All patients were withdrawn from the colchicine treatment 10 days before the studies. All assessments of the patients with FMF were performed during the attack-free periods. In the group with FMF, 5 patients were female and 20 male (mean age: 29.9 ± 11.4, range: 18 – 56 years). The mean disease duration was 12.8 ± 71 years (range 2–33). The control group was formed by 25 healthy volunteers (7 women and 18 men; mean age: 29.1 ± 11.8, range: 21 – 49 years) without any evidence of disease, matched in age and sex with the patients with FMF. The healthy subjects and the patients were not habituated to smoking and/or alcohol consumption, and were diagnosed as being free from liver and kidney diseases, diabetes mellitus, peripheral neuropathy, familial hypercholesterolemia, thyroid and parathyroid diseases, and hematological, lymphoproliferative and other malignant diseases.

Laboratory assessments

All patients were evaluated in terms of a complete blood count, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) at the beginning of the study. ESR and CRP were determined according to the Westergren method and a nephelometric method (Beckman Array Protein System, USA), respectively.

Cu/Zn-SOD assay

Cu/Zn-SOD activity was assayed according to Sun et al (17). In this method, xanthine-xanthine oxidase complex produces superoxide radicals, which react with nitroblue tetrazolium (NBT) to form a farmasone compound. The Cu/Zn-SOD activity is measured at 560 nm by detecting the inhibition of this reaction. One unit of Cu/Zn-SOD is defined as the amount of enzyme causing half-maximal inhibition of NBT reduction and activity was expressed as U/L.

PON-1 assay

PON-1 activity was measured according to the method previously described (13). Diethyl-p-nitrophenylphosphate was used as a substrate in measuring. Briefly, 15 µl of serum (for each sample) was pipetted in the micro well plate, and then the enzymatic reaction was started by addition of 235 µl assay buffer, following the formation of p-nitrophenol by its absorbance at 405 nm for 3 min at 37 °C. The assay mixture contained 1.2 mM paraoxon, 1 mM $CaCl_2$, and 10 mM Tris-HCl with 1 M NaCl (pH 8.5). Enzymatic activity was calculated using the molar extinction coefficient 18000 $M^{-1} \cdot cm^{-1}$. One unit of paraoxonase activity was defined as the enzyme quantity that disintegrates 1 mmol paraoxon substrate in 1 min, and activity was expressed as units per ml of serum (U/mL).

ARE assay

Arylesterase activity was measured spectrophotometrically as previously described (13). The reaction mixture contained 1.5 mM of phenylacetate, 0.9 mM $CaCl_2$, and 9 mM Tris-HCl at pH 8.5. The reaction was initiated by addition of a 1:40 dilution of serum (in deionized water), and the increase in absorbance at 270 nm was recorded every 30 second for 3 min at 37 °C. The molar extinction coefficient of 1310 $M^{-1} \cdot cm^{-1}$ was used for calculating the activity. One unit of arylesterase activity equaled to 1 mmol of phenylacetate hydrolyzed per min, and activity was expressed as units per ml of serum (U/mL).

MDA assay

The assay was performed using the thiobarbituric acid method (18). Serum aliquots (0.2 ml) were mixed thoroughly with 0.8 ml of phosphate buffered saline (pH 7.4) and 0.025 ml of butylated hydroxytoluene solution. After 0.5 ml of 30 % trichloroacetic acid was added, the samples were placed on ice for 2 hr and then centrifuged at 2000 x g at 25 °C for 15 min. One ml of supernatant was mixed with 0.075 ml of 0.1 mol/L EDTA and 0.25 ml of 1% thiobarbituric acid in 0.05 N sodium hydroxide. The samples were placed in boiling water for 15 min, cooled to room temperature, and the absorbance was determined at 532 nm. Total thiobarbituric acid reactive substances (TBARS) were expressed as MDA, using a molar extinction coefficient for MDA of 1.56×10^5 $cm^{-1} \cdot M^{-1}$. The results were expressed as µmol /L.

Statistical analysis

Data were processed using the SPSS 11.0 package program. The values were given as mean ± standard deviation (SD). Differences between groups were analyzed using the Mann – Whitney U test. The *p* value of less than 0.05 was considered statistically significant.

Results

The clinical features and laboratory findings of the patients and the control subjects are shown in Table 1. Demographic vari-

Tab. 1. The clinical and laboratory features of the patients with FMF and healthy controls.

	FMF group	control group	p-value
Sex (male/female)	20/5	18/7	ns
Age (years)	29.9±11.4	29.1±11.8	ns
Disease duration (year)	12.8±7.1	–	–
BMI (kg/m2)	22.2±5.3	21.7±5.04	ns
ESR (mm/h)	30.3±28.8	25.7±18.2	ns
CRP (mg/dl)	0.96±0.77	0.83±0.64	ns
Cu/Zn-SOD (U/L)	2.54±0.68	2.15±1.09	ns
PON-1 (U/ml)	36.1±20.7	38.4±23.5	ns
MDA (µmol/L)	2.43±1.87	2.16±0.97	ns

Cu/Zn-SOD: Cu/Zn-Superoxide dismutase; PON-1: paraoxonase-1; ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, BMI: body mass index; ns: not significant

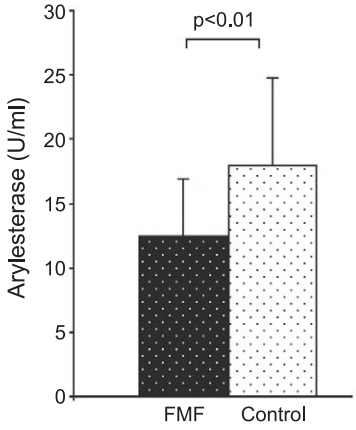

Fig. 1. The serum arylesterase activities in FMF patients and healthy controls.

ables such as age, sex and body mass index (BMI) were similar for the patients and controls ($p > 0.05$). No statistically significant difference in the ESR and CRP serum levels between the groups was observed ($p > 0.05$).

The activities of the Cu/Zn-SOD, PON-1 and ARE and MDA levels in serum were determined in the patients with FMF and the healthy subjects. No statistically significant difference in the serum Cu/Zn-SOD, MDA and PON-1 values among the study groups was observed ($p > 0.05$) (Tab. 1). However, ARE activity was significantly decreased in the patients with FMF compared to the healthy subjects ($p < 0.01$) (Fig. 1).

Discussion

FMF is an autosomal recessive disease, characterized by recurrent episodes of fever, peritonitis and/or pleuritis. Oxidant stress constitutes a serious pathophysiological factor for a wide variety of connective tissue disorders, such as FMF. The pathogenic mechanism of chronic inflammation is associated with an increased production of ROS and free radicals (superoxide anion and hydrogen peroxide) (11). Oxidative stress situations are characterized by an increase in the concentration of free radicals. Increased levels of cellular oxidative stress can result from many factors, including exposure to alcohol, medications, inflammation, trauma, cold, infections, poor diet, toxins, radiation or excessive physical activity (19).

The overexpression of the antioxidant enzymes may block ROS and free radical-induced events, reducing the inflammatory response and tissue destruction in joints (7). Another important result of increased free radical and ROS is lipid peroxidation. A complex antioxidant defense system has evolved in humans, and the antioxidant enzymes play an important role in protecting cellular homeostasis. Some evidence indicates an increased oxidative stress in patients with FMF (14, 20). However, controversial results have been obtained in studies of acute phase reactants during attack-free periods (21 – 23). In our study, no significant differences in serum ESR and CRP levels between patients with FMF and the control group were observed.

Abnormalities in the antioxidant status and lipid peroxidation in the serum and tissues in patients with rheumatic disease have been reported in the medical literature (24, 25). The results remain complicated and controversial. Several reports revealed an increase in serum Cu/Zn-SOD in patients with rheumatic disease (26 – 28). PON-1 and ARE enzymes, which are located on HDL, have lipophilic antioxidant characteristics. Both have been shown to be functions of single enzymes (29). In our research, we did not find a study involving serum Cu/Zn-SOD, PON-1 and ARE levels in patients with FMF. In the present study, while PON-1 activity was not different from that in the control subjects, the serum ARE activities were significantly lower in the patients with FMF compared to the control group.

In literature, the PON-1 activity related article reported different results. In Mackness et al's study, PON-1 activity in Type II DM was higher than in advanced retinopathy in Type II DM (30). According to the Bodolay et al study, PON-1 activity in patients with mixed connective tissue disease was lower than in the control group (31). In a study conducted in patients with rheumatoid arthritis, serum PON-1 activity was lower compared to the control group (32). However, according to Gullulu et al's study, the PON-1 activity in patients with glomerulonephritis was lower than in the control groups while no significant difference in serum ARE activity was observed between the groups (33).

Tanimoto et al found a decreased PON-1 and ARE activity in the serum of RA (34). Erdem et al did not observe significant differences between patients with ankylosing spondylitis and the controls in terms of serum PON-1 or ARE levels (35). We found only a study investigating MDA levels in patients with FMF. Gurbuz et al observed increased plasma MDA levels in patients with FMF compared to the healthy controls (14). In contrast, the serum MDA levels in patients with FMF during attack-free periods were not different from those in the control group in our study.

In conclusion, abnormalities in the antioxidant defense system and lipid peroxidation may define patients with FMF. Especially, decreased ARE activity may play a role in the subclinical inflammation process in FMF disease during attack-free periods. However, further long-term studies on the subject are needed to explore altered lipid peroxidation and antioxidant defense mechanisms in patients with FMF.

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