Ailesel Akdeniz Ateşi Hastalarında Plazma MDA Düzeyi, GSH-Px Aktivitesi ve Nitrit/Nitrat Düzeyi

Plasma MDA Levels, GSH-Px Activities and Nitrite/Nitrate Levels in Patients with Familial Mediterranean Fever

Ailesel Akdeniz Ateşi ve Oksidatif Stres / Familial Mediterranean Fever and Oxidative Stress

Ahmet Tüzün[1], Bülent Değertekin[1], Zeki Yeşilova[1], İlkin Naharcı[2], Ayşe Eken[3], Ahmet Aydın[3], Ahmet Uygun[1], Ahmet Sayal[3].

[1]Department of Gastroenterology, Gülhane Military Medical Academy, Ankara, Turkey.

[2]Department of Internal Medicine, Gülhane Military Medical Academy, Ankara, Turkey.

[3]Department of Pharmaceutical Sciences, Gülhane Military Medical Academy, Ankara, Turkey.

Özet

Amaç

Endotel fonksiyonlarını olumsuz yönde etkilediği bilinen genetik hastalıklardan birisi de Ailesel akdeniz ateşi (FMF)'dir. Bu çalışmanın amacı, FMF hastalarında oksidatif stres ve nitrik oksit (NO) ile ilişkili parametrelerin araştırılmasıdır.

Gereç ve Yöntemler

Türk toplumundan 41 adet FMF'li hasta (38 erkek, 3 kadın) ve 32 adet kontrol birey (29 erkek, 3 kadın) seçildi. Tüm bireylerde glutatyon peroksidaz (GSH-Px), malondialdehit (MDA) ve nitrite/nitrate (NO_2^-/NO_3^-) düzeyleri ölçüldü.

Bulgular

MDA ve NO_2^-/NO_3^- düzeyleri hastalığın akut fazında kontrolden anlamlı olarak yüksekti ($p<0.001$). Akut fazda ve kontrol bireylerindeki GSH-Px aktivitesinde anlamlı bir fark bulunmadı. MDA ve NO2-/NO3- düzeyleri ataksız dönemde de kontrolden anlamlı olarak yüksekti ($p<0.001$). GSH-Px aktivitesine göre ataksız dönem ve kontrol bireyleri arasında anlamlı bir fark bulunmadı.

Sonuç

Bulgularımız, FMF hastalarında oksidatif durum ve NO metabolizmasının etkilendiğini göstermektedir. Ataksız dönemdeki yüksek MDA ve NO_2^-/NO_3^- düzeyleri, lipit peroksidasyonun giderilmesi ve NO yolağı aktivasyonunun normale dönmesi için belirli bir zamana ihtiyaç duyulması nedeniyle olabilir.

Anahtar Kelimeler

Ailesel Akdeniz Ateşi, Oksidatif Stres, Nitrik Oksid.

Abstract

Aim

One of the genetic diseases known to adversely affect endothelial function is familial Mediterranean fever (FMF). The purpose of this study is to investigate the status of oxidative stress and nitric oxide (NO) related parameters in patients with FMF.

Material and Methods

41 subjects with FMF (38 male - 3 female) and 32 healthy control subjects (29 male - 3 female) were selected from Turkish population. Glutathione peroxidase (GSH-Px), malondialdehyde (MDA), and nitrit/nitrat (NO_2^-/NO_3^-) levels were investigated in all subjects.

Results

MDA and NO_2^-/NO_3^- levels of patients in the acute phase were significantly higher than those of control ($p< 0.001$). No significant difference was found in GSH-Px activities between in the acute phase patients and the control subjects. MDA and NO_2^-/NO_3^- levels of patients in the attack-free period were also significantly higher than those of control ($p< 0.001$). No significant difference was found between the patients in the attack-free period and the control according to GSH-Px activities.

Conclusions

Our findings point out that oxidative status and NO metabolism is affected in FMF patients. High MDA and NO_2^-/NO_3^- levels in the attack-free phase of patients may be due to the certain period of time needed for the relief of lipid peroxidation and the reversal of NO pathway activation.

Keywords

Familial Mediterranean Fever, Oxidative Stress, Nitric Oxide.

DOI: 10.4328/JCAM.10.1.11 **Received:** 27.07.2009 **Accepted:** 28.08.2009 **Printed:** 01.01.2010 J.Clin.Anal.Med. 2010;1.1:1-5

Corresponding author: Ahmet Tüzün, M.D., Associate Prof., Gülhane Military Medical Academy, Department of Gastroenterology, 06018 Etlik, Ankara, TURKEY, **Phone:** +90 312 3044042, **GSM:** +90 532 5547446, **Fax:** +90 312 3042010, **E-mail:** ahmettuzun@yahoo.com

Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by recurrent attacks of fever and inflammation in the peritoneum, synovium, or pleura, accompanied by abdominal pain. It is most prevalent among Armenians, Sephardic Jews, Levantine Arabs, and Turks. Blood tests reveal a high erythrocyte sedimentation rate (ESR), leukocytosis, and a high fibrinogen level. Molecular genetic testing for MEFV gene (chromosomal locus 16p13.3) usually confirms the diagnosis.

Endothelial cell dysfunction plays an important role in the development and progression of cardiovascular and other diseases. There are some genetic diseases known to adversely affect endothelial function. One of them is FMF [1]. Endothelial dysfunction results from a complex interplay between genetic and environmental factors.

Nitric oxide (NO) is the key endothelium-derived relaxing factor, which plays a pivotal role in the maintenance of vascular tone and reactivity [2]. NO is synthesized from L-arginine under the influence of nitric oxide synthase (NOS) in many cell types, including platelets, fibroblasts, neuronal cells, endothelial cells, macrophages and neutrophils. NO is a nonstable radical and is converted to a more stable product, nitrite/nitrate (NO_2^-/NO_3^-) anion, in a very short time. However, excess NO can exert cytotoxic and cytostatic effects [3,4]. Reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot-}$), H_2O_2 and peroxynitrite can be produced in blood vessels by certain drug agents and pathological conditions inducing (or accentuating) endothelial cell dysfunction. Addition of peroxynitrite to biological fluids leads to nitration of aromatic amino acid residues such as nitrotyrosine. The presence of such residues indicates peroxynitrite mediated (i.e., NO-dependent) cell damage in vivo (3). Endogenous defenses against ROS include antioxidant enzymes such as: glutathione peroxidase (GSH-PX), catalase (CAT) and superoxide dismutase (SOD) [5]. Lower levels of essential antioxidants in the circulation have been found to be associated with an increased risk of pathology. So far, there is not a considerable body of evidence on the status of both antioxidative systems and NO pathways in patients with FMF. Therefore, we aimed to demonstrate the status of oxidative stress and nitric oxide related parameters by investigating plasma GSH-Px activities, plasma levels of MDA and NO_2^-/NO_3^- in patients with FMF. SOD and CAT activities could not study since our samples are plasma.

Material and Methods

The study group included 41 patients with FMF (38 male and 3 female with mean age of 21; age range, 20-37). They were chosen among patients who were admitted to the outpatient clinic with the suspicion of FMF and experienced an acute attack during their observation period. The patients were not receiving colchicine treatment during this observation period.

After the occurrence of an attack, the patients were followed for clinical and laboratory findings for three consecutive days. Patients fulfilling Tel Hashomer criteria were included in the study [6]. The patients were investigated both in the active and in the attack-free stages in terms of study parameters to find out the differences between acute attack and attack-free periods, if any. The interval between the admittance (obtained blood sample referred to attack-free stage) and an acute attack was ranging between 5 to 33 days (median: 15 days). The disease activity was evaluated by the presence of abdominal pain, fever and appropriate laboratory investigations such as the neutrophil count, ESR, fibrinogen and serum CRP.

A total of 32 healthy volunteers, without clinical or laboratory evidence of any disease, were included in the study, as controls (29 male and 3 female, mean age 22; age range 20-39). Informed consent was obtained from all subjects. Plasma GSH-Px, MDA, and NO_2^-/NO_3^- levels were investigated in patients with FMF and control subjects.

GSH-Px activity measurement

GSH-Px activity in plasma was measured by the method of Pleban et al [7]. The reaction mixture was 50 mmol/L tris buffer, pH 7.6 containing 1 mmol/L of Na_2EDTA, 2 mmol/L of reduced glutathione (GSH), 0.2 mmol/L of NADPH, 4 mmol/L of sodium azide and 1000 U of glutathione reductase. 50 µL of plasma and 950 µL of the reaction mixture were mixed and incubated for 5 min. at 37°C. Then the reaction was initiated with 8.8 mmol/L H_2O_2 and the decrease in NADPH absorbance was followed at 340 nm for 3 min. Enzyme activity was reported in U/L in plasma.

MDA assay for plasma

Lipid peroxidation product MDA was estimated by measurement of thiobarbituric acid reactive substances (TBARS) in the supernatant by the method as described by Ohkawa et al. [8]. After the reaction of MDA with thiobarbituric acid, the reaction product was followed spectrophotometrically at 532 nm, using tetramethoxypropane as a standard. The results are expressed as nmol/ml.

NO_2^-/NO_3^- level measurement

Plasma NO_2^-/NO_3^- concentrations were determined by using the Griess reaction according to Tracey et al with minor modification [9]. The reaction mixture consisted of reduced nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD) and nitrate reductase. After incubation of plasma samples with reaction mixture, Griess reagent (a 1:1 mixture of 1% sulfanilamide in 5% H_3PO_4 and 0.1% N-[1-naphtyl]-ethylenediamine) was added to the samples. After incubating for 10 min, the absorbance was measured spectrophotometrically at 540 nm. The nitrite/nitrate concentrations in the samples were calculated from standard curve. Nitrite/nitrate (NO_2^-/NO_3^-) level of plasma was expressed as µmol/L.

Statistical analysis

The data gathered from the study were evaluated using Kolmogorov-Smirnov test to find out the distribution characteristics. It was found that the data distribution showed a nonparametric pattern. That's why nonparametric statistical methods were preferred for statistical evaluations. Unpaired data were compared using Mann-Whitney U test whereas paired data and repeated measurements were compared using Wilcoxon-Signed Ranks test. Categorical variables were evaluated using Chi-Square test. Exact test was applied as appropriate. α was set to 0.05 during the calculations and calculated p value less than 0.05 was accepted as statistically significant. Data were expressed as median (interquartile range) within the text.

Results

There was no significant age difference between patient and control groups (z= -1.49, p= 0.136). No significant sex difference was noted between patient and control groups (x^2= 0.101, p= 1.00). Table 1 demonstrates demographic characteristics of patients and control group associated with plasma GSH-Px, MDA, and NO_2^-/NO_3^- levels.

Plasma MDA and NO_2^-/NO_3^- levels of patients group in the acute phase were significantly higher than those of control group (p< 0.001 and p< 0.001, respectively). No significant difference was found in plasma GSH-Px levels between the acute phase in patients and the control subjects (p= 0.16). Figure-1 demonstrates the results of plasma MDA, NO_2^-/NO_3^- and GSH-Px in form of bars graphs.

Plasma MDA and NO_2^-/NO_3^- levels of patient group in the attack-free period were also significantly higher than those of control group (p< 0.001 and p< 0.001, respectively). No significant difference was found between the attack-free period in patients and the control group as plasma GSH-Px levels were taken into consideration (p= 0.601). No significant difference was observed in plasma MDA and NO_2^-/NO_3^- levels between the acute phase and attack-free period of patients. We found a significant difference in plasma GSH-Px levels between the acute phase and attack-free period of patients (z= -2.031, p= 0.043).

Table 1. Demographic characteristics, plasma GSH-Px, MDA, and NO_2^-/NO_3^- levels of patient and control subjects

	N	Sex (M/F)	Age	GSH-Px (U/L)	MDA (nmol/ml)	NO_2^-/NO_3^- (µmol/L)
Patients	41	38 M, 3 F	21 (2)	-	-	-
Active		-	-	1.2700 (0.1800)	0.4800 (0.2350)	106.0000 (50.5000)
Attack-free		-	-	1.2100 (0.1500)	0.4900 (0.2000)	106.0000 (91.0000)
Controls	32	29 M, 3 F	22 (3.75)	1.2300 (0.1650)	0.1100 (0.0450)	71.0000 (16.7500)

FMF: familial Mediterranean fever; GSH-Px: Glutathione peroxidase; MDA: Malondialdehyde; NO_2^-/NO_3^-: Nitrite/nitrate.

Discussion

Familial Mediterranean fever begins in childhood with paroxysmal attacks of abdominal pain and fever accompanied by peritonitis, pleuritis, and synovitis. During the acute phase, there is a massive influx of polymorphonuclear neutrophils into the serosal membranes. Diminished activity of a chemotactic inhibitor in synovial fluids from patients with FMF has been proposed as a reason for the neutrophil influx into serosal cavities [10].

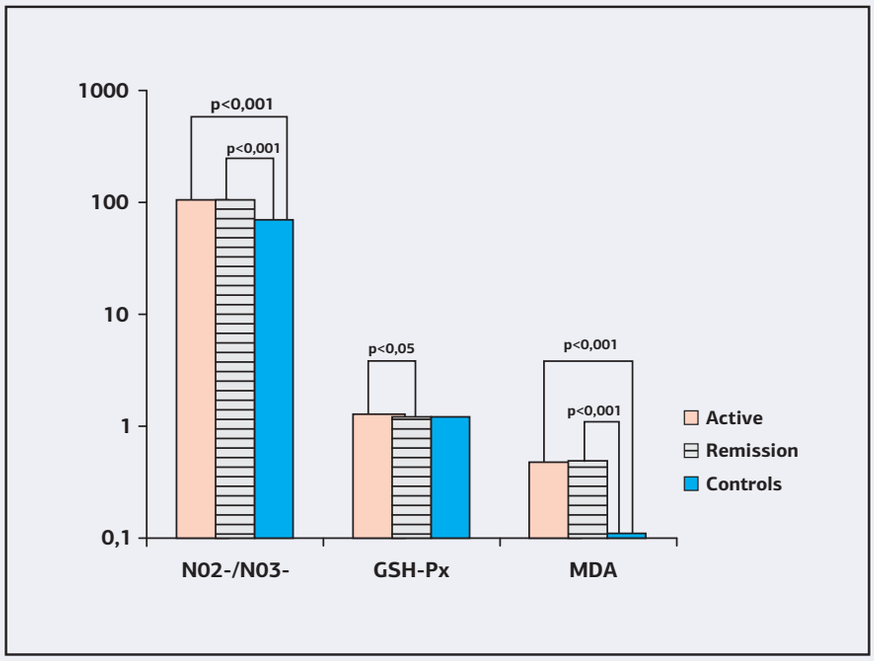

Figure 1. The results of plasma NO_2^-/NO_3^-, GSH-Px and MDA in form of bars graphs

Increased chromosome breakage is observed in patients with FMF. Their plasma contains clastogenic material inducing chromosome damage in cells from healthy persons. It is proposed that increased oxyradical generation by activated polymorphonuclear cells in blood and serosal fluids of these patients leads to the formation of a clastogenic factor, as it is observed in other chronic inflammatory diseases. Also similar to these diseases, the clastogenic effects are prevented by superoxide dismutase and partially by inhibitors of arachidonic acid metabolism [11]. Although FMF is not an autoimmune disease, four vasculitidies (Henoch Schonlein purpura, polyarteritis nodosa, protracted febrile myalgia, Behçet's disease) have been encountered in about 3% of FMF patients [12]. The prevalence of Behçet's disease (BD) was found to be higher in FMF patients than in populations known to be rich in BD [13]. To our knowledge, there has been no human clinical research done on GSH-Px levels in patients with FMF to date. Erkilic et al. found significantly decreased plasma and erythrocyte superoxide dismutase (SOD) and GSH-Px activities, and significantly increased plasma TBARS levels in patients with BD in comparison with controls [14]. Karaguezyan et al. found increased plasma MDA levels in acute phase and attack-free period of FMF patients [15]. In this study, our MDA results are in agreement with those of Karaguezyan et al.

Nitric oxide is an important mediator in inflammatory and autoimmune-mediated tissue destruction. It is also implicated in the pathogenesis of many diseases, including diabetes [9], arthritis [16], chronic inflammation [17], cancer [18], and others. Panossian et al. reported that plasma NO level in active FMF patients were lower than those of inactive patients and the healthy controls [19]. And also, this finding was reported as quite uncommon for chronic inflammatory disorders, characterized by elevated plasma NO level by Panossian et al. They suggested that the decreasing of NO in attacks might be a defense compensatory response in order to decrease the inflammation. They said, however, that induced NO can suppress the inflammatory response by down-regulating pro-inflammatory cytokine release and inhibiting neutrophil accumulation. Their alternative suggestion was that decreased formation of NO in FMF attack would increase the production of proinflammatory cytokines (i.e., IL-6), which are elevated in FMF attacks. In the present study, contrary to Panossian et al., we demonstrated that nitrite/nitrate (the end product of NO) levels were significantly higher in the patients group than that of the healthy controls. This finding pointed out that endothelial function could be activated as a protective mechanism. But it is considered that increased NO can be toxic via peroxynitrite formation. This pathway is considered to be still active in attack-free period and it can improve with time. In our previous study, we suggested the continuity of cytokine activation in these patients on the elevation sIL-

2R and IL-6 levels both before and during the attack compared to healthy control group [20].

In our study, increased GSH-Px activity in the acute phase of disease indicated a high radical generation. In parallel with this result, MDA levels were also increased. However GSH-Px activity increased, it did not prevent MDA production. The decreasing GSH-Px levels in attack-free period to control levels can be explained as a relative decrease in radical generation. But, MDA levels still remain high in the attack-free period. So we can conclude that certain amount of time is needed for the improvement of lipid peroxidation.

As a result, we conclude that oxidative status and NO pathway are affected in FMF patients. High plasma MDA and NO_2^-/NO_3^- levels in the attack-free phase of patients may be due to the certain period of time needed for the relief of lipid peroxidation and the reversal of NO pathway activation.

Acknowledgements

The authors thank to Mustafa Turan for his comments on manuscript.

References

1. Butany JW, Verma S, Leask RL, Mohsen B, Asa SL. Genetic abnormalities of the endothelium. Microsc Res Tech 2003;60:30-37.
2. Cantor WJ, Butany J, Iwanochko M, Liu P. Restrictive cardiomyopathy secondary to Fabry's disease. Circulation 1998;98:1457-1459.
3. Kaur H, Halliwell B. Evidence for nitric oxide-mediated oxidative damage in chronic inflammation. Nitrotyrosine in serum and synovial fluid from rheumatoid patients. FEBS Lett 1994;350:9-12.
4. Biswas SK, Bhelwa AP, Upadhyay AU, George A, Nath N. Status nitric oxide free radicals in diabetic neutrophils: effect of diabetic serum factor on generation of these species in normal neutrophils and their relation to lysosomal degranulation. Indian J Biochem Biophs 1993;30:293-296.
5. Ray G, Batra S, Shukla NK, Deo S, Raina V, Ashok S, Husain SA. Lipid peroxidation, free radical production and antioxidant status in breast cancer. Breast Cancer Res Treat 2000;59(2):163-70.
6. Livneh A, Langevitz P, Zemer D, Zaks N, Kees S, Lidar T, et al. Criteria for the diagnosis familial Mediterranean fever. Arthritis Rheum 1997;40:1879-1885.
7. Pleban PA, Munyani A, Beachum J. Determination of selenium concentration and glutathione peroxidase activity in plasma and erythrocytes. Clin Chem 1982;28:311-316.
8. Ohkawa H, Ohiski N and Yagi K. Assay for lipid peroxides in animals and tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-358.
9. Aydin A, Orhan H, Sayal A, Ozata M, Sahin G, Isimer A. Oxidative stress and nitric oxide related parameters in type II diabetes mellitus: effects of glycemic control. Clin Biochem 2001;34:65-70.
10. Matzner Y, Brzezinski A. C5a-inhibitor deficiency in peritoneal fluid from patients with familial Mediterranean fever. N Engl J Med 1984;311:287-290.
11. Emerit I, Arutyunyan R, Sarkisian T, Mejlumian H, Torosian E, Panossian AG. Oxyradical-mediated chromosome damage in patients with familial Mediterranean fever. Free Radic Biol Med 1993;15:265-271.
12. Mor A, Gal R, Livneh A. Abdominal and digestive system associations of familial Mediterranean fever. Am J Gastroenterol 2003;98:2594-2604.
13. Schwartz T, Langevitz P, Zemer D, Gazit E, Pras M, Livneh A. Behçet's disease in familial Mediterranean fever: characterization of the association between the two diseases. Semin Arthritis Rheum 2000;29:286-295.
14. Erkilic K, Everekliogluc C, Cekmen M, Ozkiris A, Duygulu F, Dogan H. Adenosine deaminase enzyme activity is increased and negatively correlates with catalase, superoxide dismutase and glutathione peroxidase in patients with Behçet's disease: original contributions/clinical and laboratory investigations. Mediators Inflamm 2003;12:107-116.
15. Karaguezyan KG, Haroutjunian VM, Mamiconyan RS, Hakobian GS, Nazaretian EE, Hovsepyan LM, et al. Evidence of oxidative stress in erythrocyte phospholipid composition in the pathogenesis of familial Mediterranean fever (periodical disease). J Clin Pathol 1996;49:453-455.
16. Farrell AJ, Blake DR, Palmer RM, Moncada S. Increased concentrations nitrite in synovial fluid and serum samples suggest increased nitric oxide synthesis in rheumatic diseases. Ann Rheum Dis 1992;51:1219-1222.
17. Nussler AK, Billiar TR. Inflammation, immunregulation, inducible nitric oxide synthesis. J Leukoc Biol 1993;54:171-178.
18. Bakan E, Taysi S, Polat MF, Dalga S, Umudum Z, Bakan N, Gumus M. Nitric oxide levels and lipid peroxidation in plasma of patients with gastric cancer. Jpn J Clin Oncol 2002;32:162-166.
19. Panossian AG, Hambartsumyan M, Panosyan L, Gabrielyan E, Amaryan G, Wikman G. Plasma Nitric oxide level in familial Mediterranean fever. 3rd International conference on familial Mediterranean fever and hereditary inflammatory disorders, September 23-27, 2002. Le Grande Motte, France. Oral communication: B4.
20. Bagci S, Toy B, Tuzun A, Ates Y, Aslan M, Inal A, Gulsen M, Karaeren N, Dagalp K. Continuity of cytokine activation in patients with familial Mediterranean fever. Clin Rheumatol 2004;23:333-337.