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Original Article

Evaluation of Superoxide Dismutase and Glutathione Peroxidase Enzyme Polymorphisms in Familial Mediterranean Fever Patients

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ABSTRACT

Familial Mediterranean Fever (FMF) is a fairly common inflammatory disease in communities with mediterranean origin. It is characterized with autosomal recessive, recurrent short-term episodes of fever, peritoneal, pleural, synovial membrane involvement and skin lesions. The aim of the present study was to investigate possible associations between FMF and Ala-9Val polymorphism of MnSOD and Pro198Leu polymorphism of GPx1. The study included 129 FMF patients who has mutations (E148Q, P369S, F479L, M680I(G/C), M680I(G/A), I692del, M694V, M694I, K695R, V726A, A744S, R761H) in the heterozygous or homozygous form and 95 healthy subjects. To identify MnSOD Ala-9Val and GPx1 Pro198Leu SNPs, genotyping was performed using PCR amplification, and polymorphisms were detected with hybridization probes labeled with fluorescent dyes. Genotype and allele frequencies of Ala-9Val polymorphism of MnSOD and Pro198Leu polymorphism of GPx1 were detected. The MnSOD Val allele frequency is 132 (51.16%) in the FMF and 115 (60.52%) in the control group (p<0.05). The GPx1 Leu allele is 83 (32.17%) in the FMF and 61 (32.11%) in the control group (p=0.988). No significant differences were found between genotype frequencies of GPx1 and MnSOD polymorphisms. According to our findings MnSOD Val allele may be a genetic factor involved in the pathogenesis of FMF. The fact that there are only few studies in literature, we need more patients, other enzyme levels and works about polymorphism to support our study.

Key words: *FMF, SOD, GPx, polymorphism.*

Ailesel Akdeniz Ateşi Hastalarında Süperoksid Dismutaz ve Glutatyon Peroksidaz Enzim Polimorfizmlerinin Değerlendirilmesi.

ÖZET

Ailevi Akdeniz ateşi (Familial Mediterranen Fever-FMF) Akdeniz kökenli topluluklarda oldukça sık görülen otozomal resesif geçişli, tekrarlayıcı kısa süreli ateş atakları, periton, plevra, sinovyal zar tutulumu ve cilt lezyonları ile karakterize enflamatuar bir hastalıktır. Bu çalışmanın amacı, FMF ve MnSOD Ala-9Val polimorfizmi ve GPx1 Pro198Leu polimorfizmi arasındaki olası ilişkiyi araştırmaktır.Çalışma heterozigot ya da homozigot şeklinde (E148Q, P369S, F479L, M680I (G/C), M680I (G/A) I692del, M694V, M694I, K695R, V726A, A744S, R761H dahil) mutasyona sahip 129 FMF hastası ve 95 sağlıklı denek içermektedir. MnSOD Ala-9Val ve GPx1 Pro198Leu SNP'leri tespit etmek için, genotipleme PCR amplifikasyonu kullanılarak gerçekleştirilmiştir ve polimorfizmler floresan boyalar ile etiketlenmiş hibridizasyon probları ile tespit edilmiştir. MnSOD Ala-9Val ve GPx1 Pro198Leu polimorfizminin genotip ve allel frekansları tespit edilmiştir. FMF'de MnSOD Val allel frekansı 132 (%51.16) kontrol grubunda 115 (%60.52) (p <0.05)'dir. FMF'de GPx1 Leu alleli 83 (%32.17) kontrol grubunda 61 (32.11%) (p= 0.988)'dir. GPx1 ve MnSOD polimorfizmlerinin genotip frekansları arasında anlamlı bir farklılık bulunamamıştır. Bulgularımıza göre MnSOD Val alleli FMF patogenezinde genetik bir faktör olabilir. Ancak literatürde az sayıda çalışma olmasından dolayı çalışmayı desteklemek için daha fazla hastaya, diğer enzim düzeylerinin ve polimorfizmlerinin çalışılmasına ihtiyaç vardır.

Anahtar Kelimeler: *FMF, SOD, GPx, polymorphism*

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INTRODUCTION

Familial Mediterranean Fever (FMF) is an autosomal recessively inherited inflammatory disease characterized with recurrent short-term fever attacks, peritoneum, pleura, synovial membrane involvement, and skin lesions (1).The most significant pathologic feature in FMF is recurrent and non-infective acute inflammatory reactions in serosal membranes. It was presented that the most frequently encountered inflammatory cell in inflammation areas during the attacks are neutrophils (2). The pain table in FMF disease is created when lysosomal enzyme activation and cell membrane integrity disappears and neutrophil degranulation increases (3). In FMF patients, monocyte and oxidative bursts in neutrophils can be observed in periods without attacks, as well (4).

Superoxide dismutase (SOD) and glutathione peroxidase (GPx) are antioxidants with enzymatic structure (5). The duty for SOD enzyme in the antioxidant system is to turn superoxide radical into hydrogen peroxide. It is three defined isoforms for which the genomic structure is known. One of these are SOD2 (MnSOD) isoform within mitochondria matrix (6). Single nucleotide polymorphism (SNP) that emerged as result of cytosine nucleotide's displacing the thymine nucleotide (T□C) was determined in 47th position coding the signal sequence in SOD2 gene. This causes changes in direction of enzyme towards mitochondria, localization and function, accordingly. This single-point mutation is called as SOD2 Ala16Val or SOD2 Ala-9Val (7,8).

GPx are enzymes involved in the reduction reaction of hydrogen peroxide (9). Some polymorphisms have been identified on the GPx1 gene. One of these is C□T polymorphism observed upon codon 198 which is located on exon 2. CCC chain coding proline amino-acid turns into CTC chain coding leucine amino-acid through cytosine's displacing by thymine on the 593rd nucleotide upon codon 198 (10). It has been reported that leucine-amino-acid's infecting into enzyme structure instead of proline affects selenium element's linking to the enzyme and the enzyme activity decreased through the changes occurring in the structure of enzyme (11). Together with the other antioxidants GPx prevents the damage of phagocytic cells as result of free radical peroxidation during the respiratory burst (12). Decrease at GPx activity causes increase at H2O2 and severe cell damage (13,14).

In the light of these information, we tried to prove to what frequency SOD2 (Ala16Val) and GPx1 (Pro198Leu) polymorphisms that could be observed in the structure of enzyme emerged affecting the activities of SOD and GPx1 enzymes which are the antioxidant enzymes that have not been analyzed in detailed studies before, and the level of this response with the FMF disease having the most frequently encountered mutations.

MATERIAL AND METHODS

Study population

In this study, we studied 224 patients who applied to the University of Gaziosmanpaşa Health Research and Application Hospital in Tokat region of Turkey with symptoms of FMF. 95 people without FMF mutation were accepted as control group, 129 people who has the FMF mutations (E148Q, P369S, F479L, M680I(G/C), M680I(G/A), I692del, M694V, M694I, K695R, V726A, A744S, R761H) in the heterozygous or homozygous form were chosen as the patient group. The hospital ethics committee approved the study, and written informed consent was obtained from each patient after the nature and purpose of the study was fully explained. All experiments were performed in accordance with the Declaration of Helsinki.

DNA Isolation

Blood specimens were drawn into EDTA containing tubes, and genomic DNA samples were extracted from the peripheral leukocytes of the collected venous blood by High Pure PCR Template Preparation Kit (Roche Molecular Biochemicals, Mannheim, Germany) according to manufacturer's instructions.

MnSOD Ala-9Val and GPx1 Pro198Leu genotyping

To identify MnSOD Ala-9Val and GPx1 Pro198Leu SNPs, genotyping was performed using PCR amplification, and polymorphisms were detected with hybridization probes labeled with fluorescent dyes (LightCycler 1.5 Real-Time PCR System, Roche Diagnostics, Mannheim, Germany). Target fragments of the human MnSOD and GPx1 genes were amplified using specific primers. To detect the MnSOD Ala-9Val polymorphism, we used 10 pmol each of the forward primer 5'-CAGCCTGCGTAGACGGTCCC-3' and reverse primer 5'-CGTGGTGCTTGCTGTGGTGC-3', and 3 pmol of the sensor probe 5'-CTCCGGCTTTGGGGTATCTG fluorescein-3' and the anchor probe 5'-LCRed640-GCTCCAGGCAGAAGCACAGCCTCC- PH-3'. To detect the GPx1 Pro198Leu polymorphism, we also used 10 pmol of the forward primer

Table 1. Distribution of MnSOD polymorphisms

SOD	FMF n:129	Control n:95	x^2	p value	OR (95% CI)
Genotype Frequency					
Ala/Ala	29(22.48%)	16(16.84%)	4.566	0.102	
Ala/Val	68(52.71%)	43(45.26%)			1.14 (0.56- 2.35)
Val/Val	32(24.80%)	36(37.89%)			1.78 (0.97- 3.28)
Allele Frequency					
Ala	126(48.83%)	75(39.47%)	3.878	<0.05	1.46 (1.01-2.14)
Val	132(51.16%)	115(60.52%)			

5'-ACTTTGAGAAGTTCC TGGTG-3' and the reverse primer 5'-TTCCTCCCTCGTAGGTTTAG-3', and 3 pmol of the sensor probe 5'-CAGACCATTGACATCGAGCCTGACATCGAA-fluorescein-3' and the anchor probe 5'-LCRed640-TGCTGTCTCAAGGGCCCAGPH-3'. The LC FastStart Master Hybridization Probes buffer (Roche Diagnostics Inc.) was used as a reaction buffer. All primers and hybridization probes were designed and synthesized by TIB MOLBIOL (Berlin, Germany). The genotypes were identified by running a melting curve with specific Tm. Wild-type MnSOD Ala exhibits a Tm of 65 ± 0.5 °C, while wild-type GPx1 Pro yields a Tm of 66 ± 0.5 °C. The allele variant MnSOD Val exhibits a Tm of 56 ± 0.5 °C, and the allele variant GPx1 Leu exhibits a Tm of 57 ± 0.5 °C. The PCR reaction was as follows: initial denaturation at 95 °C for 10 min, followed by 20 cycles at 95°C for 10 s, annealing at 60 °C (MnSOD) or 50 °C (GPx1) for 20 s, extension at 72 °C for 20 s. And a melting curve was recorded by an initial increase in temperature to 95 °C, cooling the reaction mixture to 40 °C holding for 30 s and then slowly heating it to 85 °C at 0.1 °C/s with continuous acquisition. Finally, the fluorescence signal was plotted against temperature in real time to produce melting curves for each sample.

Statistical Analyses

Analysis of the data was performed using IBM SPSS Statistics Version 20 and Epi Info 7. Quantitative variables were expressed as mean ± standard deviation, and qualitative variables were expressed as percentages. T-test was used to compare means for continuous variables. Chi-square test was applied for categorical variables and to evaluate the Hardy–Weinberg equilibrium for the distribution of the genotypes of patients and controls. P values below 0.05 were considered statistically significant.

RESULTS

The control group consists of 95 people with no FMF mutation. The FMF group however consists of 129 people with at least one FMF mutation. 14 of the 129 patients with mutation carry the homozygote and 115 the heterozygous mutation. There is no significant statistical difference in MnSOD Ala-9Val genotype frequencies between the control and patient group (Table 1). Similarly, there is no significant statistical difference in GPx1 Pro198Leu genotype frequencies between the control and FMF group (Table 2).

The Ala allele frequencies in the MnSOD Ala-9Val polymorphism is 126 (48.83%) in the FMF group and 75 (39.47%) in the control group. But the Val allele frequency is 132 (51.16%) in the FMF and 115 (60.52%) in the control group ($p<0.05$). The OR and 95% CI values were found to be 1.46 (1.01-2.14) (Table 1). The GPx1 Pro198Leu polymorphism Pro allele frequency is 175 (67.82%) in the FMF and 129 (67.89%) in the control group. The Leu allele is 83 (32.17%) in the FMF and 61 (32.10%) in the control group ($p=0.988$). OR and 95% CI values were found to be 1.01 (0.67 - 1.50) (Table 2).

Table 2. Distribution of GPx polymorphisms

GPx	FMF n:129	Control n:95	x^2	p value	OR (95% CI)
Genotype Frequency					
Pro/Pro	61(47.28%)	43(45.26%)	0.508	0,776	
Pro/Leu	53(41.08%)	43(45.26%)			1.15 (0.66- 2.02)
Leu/Leu	15(11.62%)	9(9.47%)			0.74 (0.28- 1.86)
Allele Frequency					
Leu	83(32.17%)	61(32.10%)	0.0002	0.988	1.01 (0.67- 1.50)
Pro	175(67.82%)	129(67.89%)			

DISCUSSION

The most obvious pathological feature in FMF is the chemotactic activity during episodes of increased polymorphonuclear leukocytes to turn to the affected area caused by repetitive and non-infectious acute inflammatory reactions (15). It has been shown that an increasing flow of polymorphonuclear leukocytes in the affected tissues followed by a loss of cell membrane activity which is caused by lysosomal enzymes increases neutrophil degranulation, and arachidonic acid metabolites (3,4). Increased activity of lipoxygenase enzyme and increased oxidative burst induced by arachidonic acid metabolites triggers an increase of reactive oxygen species which result in damages of various tissues, cells and DNA (16). The free oxygen radicals 8-OH-Gua, 8-OH-Ade and Fapyade, which are indicators of oxidative DNA damage in polymorphonuclear leukocytes are found significantly higher in FMF patients then in the control group (17). Oxygen radicals mediating the increase of clastogenic factors and the increase in lipid peroxidation products, show a relation with oxidative stress and FMF (18).

Although there are studies about the relationship between FMF and oxidative stress, there are only few studies about GPx enzyme levels and polymorphism. Considering the antioxidant enzyme activity in FMF patients, it has been observed that GPx activity decreased during acute attacks. In addition it has been seen that the MDA level, which is an indicator of lipid peroxidation, also increases (19-21). There are numerous studies about GPx1 Pro198Leu polymorphism in other diseases in literature (22-25). The studies about GPx1 Pro198Leu polymorphism are mostly done between malignancies, but the results about the relation between malignancies and GPx1 Pro198Leu polymorphism are conflicting. Studies about colorectal carcinoma, prostate carcinoma, schizophrenia and diseases such as tardive dyskinesia showed that the GPx1 Pro198Leu polymorphism is an important risk factor (26). Studies about GPx1 Pro198Leu polymorphism that causes GPx enzyme activity and the relation with FMF is very limited. In our study the polymorphism genotype and allele frequencies in the FMF and control group were found to be statistically irrelevant. Also Oktem et al. said that there is no significant relationship between FMF disease and GPx1 polymorphism (27). Because there are only few studies about the relationship between FMF and GPx1 polymorphism we need further studies to investigate them.

There are only few studies where the FMF disease and SOD enzyme levels and activities are investigated. It has been shown in few studies that the SOD enzyme in FMF disease is protective against oxidant agents and that during acute attack episodes the SOD activity decreases (19,28). Another study shows that the SOD enzyme activity shows no difference in FMF disease (20). As far as we know there is no study about the relationship between FMF and SOD polymorphism. However, the relationship between various diseases and MnSOD Ala-9Val polymorphisms have been examined and bladder cancer, breast cancer, Parkinson's disease and motor neuron disease have been associated with non-familial idiopathic cardiomyopathy (14). The FMF and MnSOD Ala-9Val genotype frequency was not statistically significant in our study. But if we look at the distribution of the allele frequency we see that the Val allele frequency is more distributed in the FMF group ($p<0.05$). According to our findings MnSOD Val allele may be a genetic factor involved in the pathogenesis of FMF. The fact that there are only few studies in literature, we need more patients, other enzyme levels and works about polymorphism to support our study.

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