

Evaluation of some antioxidant enzyme activities (SOD and GPX) and their polymorphisms (MnSOD2 Ala9Val, GPX1 Pro198Leu) in fibromyalgia

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Abstract. – OBJECTIVE: Fibromyalgia syndrome (FMS) is a pain syndrome in which common pain in muscle-skeletal system, sleeping disorder and fatigue symptoms coexist. The aim of the present study was to determine SOD and GPX enzyme levels in FMS as well as to investigate possible associations between FMS and Ala9Val polymorphism of MnSOD2 and Pro198Leu polymorphism of GPX1.

PATIENTS AND METHODS: The study included 127 women FMS patients and 56 healthy subjects. Total SOD and total GPX enzyme activities were determined in patient and control groups. In addition, frequencies of Ala9Val polymorphism of MnSOD2 and Pro198Leu polymorphism of GPX1 were also detected.

RESULTS: SOD enzyme activity was higher in FMS group compared to control ($p < 0.001$). GPX enzyme activity, on the other hand, was not different between FMS and control groups. No significant differences were found between genotype and allele frequencies of GPX1 and MnSOD2 polymorphisms.

CONCLUSIONS: Elevated total SOD and unchanging total GPX1 activities in FMS patients could be the reason for increased oxidative stress and lipid peroxidation in FMS. Genotype and allele frequencies of Ala9Val polymorphism of MnSOD2 and Pro198Leu polymorphism of GPX1 in FMS have been studied first time in the present study, and no associations were found between them and FMS.

Key Words:

Fibromyalgia, SOD, GPX, Oxidative stress, Polymorphism.

Introduction

Fibromyalgia syndrome (FMS) is a chronic pain syndrome accompanied by extensive pain in

body, rigidity in muscles, fatigue, poor sleep quality and cognitive difficulties as well as by syndromes such as anxiety, depression and impairment in conducting the daily activities^{1,2}. FMS incidence is higher in women than in men³. FMS etiopathogenesis have not been fully revealed yet, but central and autonomous nervous system, neurotransmitters, hormones, immune system, external stress factors and psychiatric conditions seem to be involved⁴. Recent studies have shown an association between FMS and oxidative stress⁵.

Oxidative stress is the breakdown of the balance between reactive oxygen species (ROS) and antioxidant defense system. Since ROS such as superoxide anions, hydrogen peroxide and hydroxyl radical have unpaired electrons, they are quite reactive molecules. Therefore, they damage biomolecules such as proteins, lipids and nucleic acids, resulting in various diseases⁶. Antioxidant defense system enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPX) prevents oxidative stress through inactivation of ROS. SOD enzyme eliminates damaging effects of free radicals by converting superoxide radical into oxygen and hydrogen peroxide and GPX enzyme by converting hydrogen peroxide into water^{7,8}.

Polymorphisms observed in genes encoding SOD and GPX enzymes result in decreases of activities of these enzymes. One of these polymorphisms, MnSOD2 Ala9Val, causes changes in signal sequence of the mitochondrial MnSOD enzyme and affects the transport of enzyme to mitochondria, which results in changes in function and localization of the enzyme. Pro198Leu polymorphism of GPX1 enzyme, on the other hand, causes proline to leucine change in codon 198 of GPX1 enzyme. Leucine affects the binding of se-

lenium, a necessary element for its function, to the enzyme and lowers its activity^{9,10}.

Aim of the present study was to investigate the activities of SOD and GPX enzymes in FMS patients as well as to study the association between FMS and Ala9Val polymorphism of MnSOD2 enzyme or Pro198Leu polymorphism of GPX1 enzyme.

Patients and Methods

Study Population

Study population included 125 female FMS patients who applied to Physical Medicine and Rehabilitation Polyclinics of Gaziosmanpaşa University Faculty of Medicine and had FMS diagnosis as well as 56 healthy women. Control group was composed of healthy volunteers who had no chronic diseases, normal physical examination and routine test results. In order to exclude gender specific factors, all subjects in patient and control groups were women. Study protocol was approved by Ethics Committee of Gaziosmanpaşa University Faculty of Medicine, and was carried out under Helsinki Declaration. All subjects were informed about the study and filled written consent forms.

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.

MnSOD2 Ala9Val and GPX1 Pro198Leu genotyping

To identify MnSOD2 Ala9Val and GPX1 Pro198Leu SNPs, genotyping was performed using PCR amplification, and polymorphisms were detected with hybridization probes labeled with fluorescent dyes (LightCycler 480 II Real-Time PCR System, Roche Diagnostics, Mannheim, Germany). Target fragments of the human MnSOD2 and GPX1 genes were amplified using specific primers. To detect the MnSOD2 Ala9Val polymorphism, we used 10 pmol each of the forward primer 5'-CAGCCTGCGTAGACGGTCC-3' and reverse primer 5'-CGTGGT-GCTTGCTGTGGTGC-3', and 3 pmol of the sensor probe 5'-CTCCGGCTTTGGGGTATCTG-fluorescein-3' and the anchor probe 5'-

LCRed640-GCTCCAGGCAGAAGCACAGC-CTCC-PH-3'. To detect the GPX1 Pro198Leu polymorphism, we also used 10 pmol of the forward primer 5'-ACTTTGAGAAGTTCC TG-GTG-3' and the reverse primer 5'-TTCCTCC-CTCGTAGGTTTAG-3', and 3 pmol of the sensor probe 5'-CAGACCATTGACATCGAGCCTGACATCGAA-fluorescein-3' and the anchor probe 5'-LCRed640-TGCTGTCTCAAGGGCCCCAG-PH-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 MnSOD2 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 MnSOD2 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 (MnSOD2) 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.

Measurement of SOD and GPX Enzyme Activity

For the enzymatic activity assays, whole blood specimens were taken into tubes containing EDTA. Total SOD and total GPX enzyme activities were determined based on colorimetric methods using commercial kits (Superoxide Dismutase Assay Kit, Catalog No: 706002 and Glutathione Peroxidase Assay Kit, Catalog No: 703102, Cayman Chemical Company, Ann Arbor, MI, USA). Producers' instructions were followed.

Statistical Analysis

Analysis of the data was performed using IBM SPSS Statistics Version 20 and Epi Info 7. Quantitative variables were expressed as mean $\pm$ 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 con-

Table I. Age and enzyme activities in control and FMS patient groups.

	FMS n = 127	Control n = 56	t	p
Age	43.45 ± 10.08	40.29 ± 12.01	1.722	0.088
SOD (U/ml)	16.18 ± 7.51	9.16 ± 5.20	7.295	<0.001
GPX (nmol/min/ml)	120.14 ± 30.88	113.41 ± 20.77	1.725	0.087

*Data is shown as mean ± standard deviation.

trols. *p* values below 0.05 were considered statistically significant.

Results

FMS patient group had 127 women patient while healthy control group had 56 women. Average age was 43.45±10.08 in FMS group and 40.29±12.01 in control group, and the difference was not significant (*p* = 0.088). SOD enzyme activity was significantly higher in FMS group compared to control (*p* < 0.001). GPX enzyme activity was not different between FMS and control groups (*p* = 0.087). Activities of SOD and GPX activities are given in Table I.

Frequencies of Ala9Val genotypes of MnSOD were 21.3% for Ala/Ala, 54.3% for Ala/Val, and 24.4% Val/Val in FMS groups, and 28.6% for Ala/Ala, 39.3% for Ala/Val, and 32.1% for Val/Val in control group. There was no significant difference between FMS and control groups for Ala9Val genotypes of MnSOD (*p* = 0.172). Frequencies of Pro198Leu genotypes of GPX1 were 44.1% for Pro/Pro, 40.9% for Pro/Leu, and 15% for Leu/Leu in FMS group, and 37.5% for Pro/Pro, 48.2% for Pro/Leu, and 14.3% for Leu/Leu in control group. There was no significant difference between study groups for GPX1

Pro198Leu frequencies (*p* = 0.641). Genotype frequencies are given in Table II.

Ala allele frequency was 48.4 and 48.2% in FMS and control groups. Val allele frequency, on the other hand, was 51.5 and 51.7% in FMS and control groups, respectively (*p* = 0.485; odds ratio, 1.008; 95% CI, 0.646-1.574). Frequency of Pro allele was 64.5% in FMS group and 35.4% in control group. Frequency of Leu allele was 61.6 and 38.3% in FMS and control groups, respectively (*p* = 0.294; odds ratio, 0.881; 95% CI, 0.556-1.394). The differences between FMS and control groups for MnSOD2 and GPX1 allele frequencies were not significant. Allele frequencies are given in Table III. The observed and expected frequencies of polymorphisms of MnSOD2 and GPX1 gene were in Hardy-Weinberg equilibrium in the control and patient groups.

Discussion

FMS is a pain syndrome in which extensive pain in muscle-skeleton system, sleep disorders and fatigue symptoms co-exist. Irritable bowel syndrome, chronic headache, depression, anxiety, restless leg symptom, temporomandibular dysfunction, chronic fatigue syndrome and irritable bladder syndrome are often observed in FMS¹¹. FMS fre-

Table II. Genotype frequencies.

	Genotype	FMS n (%)	Control n (%)	OR (95% CI)	p
MnSOD2	Ala/Ala	27 (21.3)	16 (28.6)	1*	0.172
	Ala/Val	69 (54.3)	22 (39.3)	0.538 (0.246-1.177)	
	Val/Val	31 (24.4)	18 (32.1)	0.980 (0.420-2.290)	
GPX1	Pro/Pro	56 (44.1)	21 (37.5)	1*	0.641
	Pro/Leu	52 (40.9)	27 (48.2)	1.385(0.699-2.744)	
	Leu/leu	9 (15)	8 (14.3)	1.123(0.427-2.951)	

*Reference genotype

Table III. Allele frequencies.

	Allele	FMS n (%)	Control n (%)	OR (95% CI)	<i>p</i>
MnSOD2	Ala	123 (48.4)	54 (48.2)	1.008 (0.646-1.574)	0.485
	Val	131 (51.5)	58 (51.7)		
GPX1	Pro	164 (64.5)	69 (61.6)	0.881 (0.556-1.394)	0.294
	Leu	90 (35.4)	43 (38.3)		

quency is higher in women, and about 80-90% of the people who have FMS diagnosis are women. FMS frequency in adult women in Turkey is 3.6%¹²⁻¹⁵. Because of higher incidence of FMS in women, patient and control groups in the current study were composed only of women subjects.

Etiology and pathogenesis of FMS have not been fully understood yet. Recent papers have pointed to the possible roles of oxidative stress in FMS pathology. Akkuş et al¹⁶ reported that malondialdehyde (MDA), an indicator of lipid peroxidation, increases and vitamin E, a vitamin that prevents lipid peroxidation, decreases in FMS patients. Eisenger et al¹⁷ studied MDA and protein carbonyl levels, an indicator of protein peroxidation. Based on their findings, MDA levels did not change while carbonyl levels increased in FMS patients. Cordero et al¹⁸ reported increased MDA levels in FMS patients. Neyal et al¹⁹ referred decreased total antioxidant (TAS) and increased total oxidant (TOS) levels in FMS patients. These studies indicate an association between FMS and oxidative stress.

Activity of SOD enzyme whose association with FMS has been investigated in the present work was the subject of previous researches. However, to our best knowledge, GPX enzyme activity in FMS has not been investigated so far. Our findings showed that SOD enzyme activity was higher in FMS patient groups compared to control group ($p < 0.001$), while GPX activity was not significantly different between FMS and control groups ($p = 0.087$) (Table I). Bağış et al²⁰ reported decreased serum SOD activity in FMS patients. Özgöçmen et al²¹ observed similar SOD enzyme activities in FMS and control groups. Bağış²⁰ and Özgöçmen²¹ studied serum SOD activity. Therefore, these studies involve cytosolic SOD activity. The current article investigated SOD activity in complete blood; thus, the enzyme levels measured in the present study represent total (cytosolic and mitochondrial) SOD activity.

It has been known that MDA, a sign of lipid peroxidation, increases in FMS patients^{16,18,20,21}.

Elevated SOD activity in FMS patients could be a response to increased oxidative stress. Higher SOD activity and similar total GPX activity in FMS patients could be due to increased exposure to oxidative stress; despite elevated SOD activity, unchanging GPX activity could result in increases in hydrogen peroxide level of the environment. It is possible that failure of GPX enzyme to convert elevated hydrogen peroxide into water could lead to the conversion of hydrogen peroxide into hydroxyl radical, a much more reactive molecule, through Haber-Weis reactions. Hydroxyl radical, in turn, could increase lipid peroxidation, which could be the reason for elevated MDA levels in FMS patients.

In this work, genotype frequencies of MnSOD2 and GPX1 were not significantly different between FMS and control groups ($p = 0.172$ and $p = 0.641$, respectively) (Table II). Similarly, there were no statistical differences for allele frequencies of MnSOD2 and GPX1 polymorphisms between FMS and control group ($p = 0.485$ and $p = 0.294$, respectively) (Table III). To our best knowledge, these polymorphisms have been studied for the first time; therefore, our findings need to be verified by additional studies.

Conclusions

Elevated total SOD activity and unchanging total GPX1 activity in FMS patients could be the reason for increased oxidative stress and lipid peroxidation observed in FMS patients. No associations were found between FMS and genotype and allele frequencies of Ala9Val polymorphism of MnSOD2 or Pro198Leu polymorphism of GPX1 which have been studied first time in FMS.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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