

The Importance of Antioxidant Enzymes and Oxidative Stress in Human Fascioliasis

İnsan Fascioliasisinde Antioksidan Enzimler ve Oksidatif Stresin Önemi

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ABSTRACT

Objective: The aim of this study was to determine the levels of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and malondialdehyde (MDA) in the patients infected with *Fasciola hepatica* and establish whether these parameters differ among the patients with fascioliasis.

Methods: The patient group consisted of 140 individuals with *F. hepatica* seropositive; the control group consisted of 140 healthy individuals who tested negative for this parasite and had no other diseases. The patient group consisted of individuals with no chronic diseases other than fascioliasis; in both the patient and the control groups, the subjects had no unhealthy habits such as smoking and alcohol consumption, etc. The blood samples taken to diagnose fascioliasis were evaluated by the ELISA method. The samples were studied according to the kit procedures for SOD, CAT, GPx and MDA markers.

Results: In this study, 43.6% of 140 individuals in the patient group infected with *F. hepatica* had CAT ($p=0.001$), 35% had GPx ($p=0.001$), 12.9% had SOD ($p=0.002$), 90.7% had MDA ($p=0.001$). There was found a statistically significant difference between the patient and the control group in terms of the positivity of these four parameters.

Conclusion: As a result, a statistically significant relationship was found between the increase in the SOD, GPx, CAT, and MDA levels and fascioliasis. The high rate of MDA revealed that oxidative stress occurred in patients with fascioliasis, resulting in an increased activity of SOD, GPx, and CAT.

Keywords: Superoxide dismutase, glutathione peroxidase, catalase, malondialdehyde, fascioliasis

ÖZ

Amaç: Bu çalışmanın amacı, *Fasciola hepatica* ile enfekte hastalarda süperoksit dismutaz (SOD), glutatyon peroksidaz (GPx), katalaz (CAT) ve malondialdehit (MDA) düzeylerini belirlemek ve fascioliasisli hastalarda bu parametrelerde farklılık meydana gelip gelmediğini ortaya koymaktır.

Yöntemler: Hasta grubu, *F. hepatica* pozitif olan 140 hastadan; kontrol grubu ise bu parazit yönünden negatif bulunan ve başka herhangi bir hastalığı bulunmayan 140 sağlıklı kişiden oluşturuldu. Hasta grubuna fascioliasis dışında herhangi bir kronik hastalığı olmayan ve hem hasta hem de kontrol grubuna sigara, alkol kullanımı olmayan kişiler dahil edildi. Hastalarda fascioliasis pozitifliğini belirlemek için alınan kan örnekleri ELISA yöntemi ile çalışıldı. Serum SOD, CAT, GPx ve MDA düzeyleri ELISA yöntemi ile değerlendirildi.

Bulgular: Bu çalışmada *F. hepatica* ile enfekte hasta grubundaki 140 kişinin %43,6'sında CAT ($p=0,001$), %35'inde GPx ($p=0,001$), %12,9'unda SOD ($p=0,002$) ve %90,7 MDA pozitifliği saptandı. Bu dört parametrenin pozitifliği açısından hasta ve kontrol grubu arasında istatistiksel olarak anlamlı fark bulundu ($p=0,001$).

Sonuç: Sonuç olarak SOD, GPx, CAT ve MDA düzeyindeki artış ile fascioliasis arasında istatistik olarak anlamlı bir ilişki saptanmıştır. Çalışmamızda MDA'nın yüksek oranda saptanması fascioliasisli hastalarda oksidatif stres oluştuğunu ve SOD, GPx ve CAT aktivitelerinde artış olduğunu ortaya koymuştur.

Anahtar Kelimeler: Süperoksit dismutaz, glutatyon peroksidaz, katalaz, malondialdehit, fascioliasis



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INTRODUCTION

Fasciola hepatica (*F. hepatica*) is a hepatic parasite that is especially common in ungulates and rarely seen in humans. The species is estimated to infect more than 17 million people worldwide. In humans, fascioliasis can present with very different clinical findings, from asymptomatic infections to severe liver cirrhosis and death. The disease is clinically characterized most commonly as abdominal pain, eosinophilia, and fever (1,2).

Oxidative stress is the situation in which potential cellular damage occurs as a result of the shift of the prooxidant-antioxidant balance in the prooxidant direction. Oxidative stress may be caused by increased free radical production and decreased antioxidant defense. For this reason, investigating the consumption of antioxidants as a biomarker of oxidative stress can be performed by evaluating the decrease in antioxidant amounts or the increase in their metabolites. The oxidants are broken down by cytoplasmic, mitochondrial, and extracellular forms of antioxidant enzyme systems, such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), with antioxidants, such as reduced glutathione (GSH), transferrin, ceruloplasmin, ascorbic acid (vitamin C), and alpha-tocopherol. Malondialdehyde (MDA), one of the main end products of lipid peroxidation, is frequently used to evaluate oxidant damage-i.e., to determine whether oxidative stress has occurred (3,4).

Migration of adults of *F. hepatica* to the host's liver is accompanied by an inflammatory reaction followed by fibrosis and cirrhosis. During the chronic stage of fasciolosis, the inflammation spreads to the bile ducts. This destructive process is always associated with chemical changes within the cell, such as increased membrane lipid peroxidation and marked suppression of the microsomal drug-metabolizing mono-oxygenase system (5).

It is noteworthy that there have been very few studies conducted on antioxidant enzymes and oxidative stress in patients with fascioliasis, both in Turkey and globally.

The aim of this study is to determine the levels of SOD, GPx, CAT, and MDA in the patients infected with *F. hepatica* and establish whether these parameters differ in the patients with fascioliasis.

METHODS

The patient group consisted of *F. hepatica* seropositive 140 cases from a variety of hospital departments. The serological diagnosis was performed with a commercial ELISA kit (DRG Diagnostics; *F. hepatica* IgG). The control group consisted of 140 healthy individuals who tested negative for the parasite and did not have any other chronic diseases. The patient group included patients without any chronic diseases other than fascioliasis. Both the patient and the control groups were formed ensuring that the

selected individuals had no adverse habits, such as alcohol consumption or smoking. The blood samples included in the study were not taken directly from the patients; blood samples taken from the patients referred to the laboratory with suspected *F. hepatica* were used.

The sera from the patient and the control groups were stored at -80 °C. Then, the sera SOD, CAT, GPx, and MDA levels were evaluated by ELISA (YLBiont, Shanghai, and Elabscience, USA). The samples were studied for SOD, CAT, GPx, and MDA markers according to the kit procedures. The positivity status was calculated according to the results recorded by the reading in the spectrophotometer at 450 nm wavelength.

Statistical Analysis

The Z(t)-test was used to compare the ratios for categorical variables. Pearson Correlation and Linear Regression Analysis were used to analyze the relationships between the variables. Statistical significance level was taken as 5% in calculations. The calculations were used the SPSS (ver:26) statistical package programs.

RESULT

The ratio numbers of the females to males in patients and the control groups were 75/65 and 90/50 respectively. The mean age of the patients was 35.2±18.6 years while in the control group was 32±16.1 years. Regarding age and gender, no statistically significant difference was observed between the two groups.

In this study, 43.6% of the 140 patients infected with *F. hepatica* had CAT ($p=0.001$), 35% had GPx ($p=0.001$), 12.9% had SOD ($p=0.002$), 90.07% had MDA ($p=0.001$). There was a statistically significant difference between the patient and the control groups in terms of the positivity of these four parameters (Table 1). MDA was also found positive in the CAT, GPx, and SOD positive patients.

DISCUSSION

The studies show that when the organism's defense (antioxidant) mechanisms against oxidative stress are insufficient, oxidative damage develops in the cells significantly disrupting the organism's functions. This disruption is reported to possibly cause an increase in the severity of the disease (3-5). In the histopathological evaluation of the mice infected with *F. hepatica*, periportal fibrous hepatitis, composed of abundant inflammatory infiltrates in portal spaces, and bile duct hyperplasia were detected. These findings were reported to be related to the host free radical production demonstrated in sera samples and the liver due to parasite infection (6). In this study, the antioxidant

Table 1. CAT, GPx, SOD, MDA positivity rates and statistical evaluation results in the patient and the control groups

Parameter	Patient group (n=140)	Control group (n=140)	p
CAT	61 (43.6%)	30 (21.4%)	0.001
GPx	49 (35%)	3 (2.1%)	0.001
SOD	18 (12.9%)	4 (2.9%)	0.002
MDA	127 (90.7%)	58 (41.4%)	0.001

CAT: Catalase, GPx: Glutathione peroxidase, SOD: Superoxide dismutase, MDA: Malondialdehyde

enzymes levels such as SOD, GPx, and CAT and to investigate oxidant damage -i.e., to determine whether oxidative stress occurs- the MDA values were tested by the ELISA method in the patient group infected with *F. hepatica* and the healthy control group.

In this study, 43.6% of the 140 patients infected with *F. hepatica* had CAT ($p=0.001$), 35% had GPx ($p=0.001$), 12.9% had SOD ($p=0.002$), 90.07% had MDA ($p=0.001$). A statistically significant difference was determined between the patient and control groups in terms of the positivity of these four parameters. Karsen et al. (7) studied total oxidant status (TOD), total antioxidant capacity (TAC), and CAT activity in 22 patients with fascioliasis and a control group consisting of 26 healthy individuals and calculated the oxidative stress index (OSI). No significant difference in CAT level was found between the two groups. However, the researchers found that the plasma levels of total TOS and OSI were significantly increased, and the TAC level was significantly lower in the patients than in the healthy controls. As a result, high oxidative stress occurred during the course of the *F. hepatica* infection. In the study by Kamel et al. (8), MDA, SOD, CAT, and GPx activities were investigated in 20 patients with chronic fascioliasis and 10 healthy individuals as the control group. They found significant differences in the four parameters between the patient and control groups. They reported that the findings of increased sera lipid peroxidation and decreased antioxidant enzyme levels in the erythrocyte samples from the patients with chronic fascioliasis indicated the presence of oxidative stress. In a study that exhibited significantly reduced activity levels of SOD, CAT, GPx, and vitamin E in patients with fascioliasis of different infection intensities compared with controls, reduced antioxidant abilities during the disease course of fascioliasis and enhanced generation of reactive oxygen species synergically superadded to the destructive effect of fascioliasis (9).

Fascioliasis has been found to cause oxidative stress not only in humans but also in animals. Benzer and Ozan (10) evaluated the effects of natural distomatosis on the MDA concentration, enzymatic antioxidants' (GPx, Cu, Zn-SOD, CAT) activity, and concentration of non-enzymatic antioxidants (reduced GSH, vitamin C, β -carotene) in sheep liver. In the study of the MDA concentration, the GPx activity, and the ALT and AST sera activities in the sheep naturally infected with *F. hepatica*, *F. gigantica* and *Dicrocoelium dentriticum* were found significantly higher than the control group. Cu/Zn-SOD, CAT activities, GSH, and vitamin C concentrations were found significantly lower than in the control group. Saleh (11) took liver and blood samples from 27 sheep infected with *F. hepatica* and 20 healthy sheep as a control group and evaluated their plasma MDA level and antioxidant status. The study reported that the MDA level in the parasite-infected group showed a positive correlation with the parasite load; there was a statistically significant difference between the experimental and the control groups in terms of MDA level. In a study conducted by Kuzucu (12), it was stated that there was a statistically significant difference between the CAT levels of cattle with positive *F. hepatica* and negative cattle with ELISA and stool examination methods.

Kolodziejczyk et al. (5) evaluated the SOD, GPx, glutathione reductase (GSSG-R), and CAT activity in mice experimentally infected with *F. hepatica* 4, 7 and 10 weeks after infection. The research determined that there were decreases in the SOD, GSSG-R and GPx activities and increases in the amount of CAT

in the mice; the antioxidant sufficiency of rat liver decreased during fascioliasis; during infection, reactive oxygen species acted synergistically to increase their interaction with hepatocyte components and stimulate changes in their structure and function.

As a result of this study, a statistically significant relationship was found between the increase in SOD, GPx, CAT, and MDA levels and fascioliasis. The fact that MDA was detected at a high rate revealed that oxidative stress occurred with an increase in the SOD, GPx, and CAT activities in the patients with fascioliasis.

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* Ethics

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* Authorship Contributions

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REFERENCES

1. Mas-Coma MS, Esteban JG, Bargues MD. Epidemiology of human fascioliasis: a review and proposed new classification. Bull World Health Organ 1999; 77: 340-6.
2. Caravedo MA, Cabada MM. Human fascioliasis: current epidemiological status and strategies for diagnosis, treatment, and control. Res Rep Trop Med 2020; 11: 149-58.
3. Callahan HL, Hazen-Martin D, Crouch RK, James ER. Immunolocalization of superoxide dismutase in *Dirofilaria immitis* adult worms. Infect Immun 1993; 61: 1157-63.
4. Eken A. Rat Kan ve doku örneklerinde oksidatif stres parametreleri. Journal of Clinical and Analytical Medicine 2012; (Suppl): 69-73.
5. Kolodziejczyk L, Siemieniuk E, Skrzydlewska E. Antioxidant potential of rat liver in experimental infection with *Fasciola hepatica*. Parasitol Res 2005; 96: 367-72.
6. Bottari NB, Mendes RE, Lucca NJ, Schwertz CI, Henker LC, Olsson DC, et al. Oxidative stress associated with pathological lesions in the liver of rats experimentally infected by *Fasciola hepatica*. Exp Parasitol 2015; 159: 24-8.
7. Karsen H, Sunnetcioglu M, Ceylan RM, Bayraktar M, Taskin A, Aksoy N, et al. Evaluation of oxidative status in patients with *Fasciola hepatica* infection. Afr Health Sci 2011; (Suppl 1): S14-8.
8. Kamel HH, Sarhan RM, Saad GA. Biochemical assessment of oxidative status versus liver enzymes in patients with chronic fascioliasis. J Parasit Dis 2015; 39: 628-33.

9. El Shazly AA, Hegazi MA, Abd Raboo MA, Sarhan OHM, Hafez EN. Impact of human fascioliasis on oxidative stress. *Journal of Nuclear Technology in Applied Science* 2014; 2: 295-303.
10. Benzer F, Ozan ST. Lipid peroxidation, antioxidant enzymes and levels of nitric oxide in sheep infected with *Fasciola hepatica*. *Türk J Vet Anim Sci* 2003; 27: 657-61.
11. Saleh MA. Circulating oxidative stress status in desert sheep naturally infected with *Fasciola hepatica*. *Vet Parasitol* 2008; 154: 262-9.
12. Kuzucu S. Determination of antioxidant level in naturally infective cattle with *Fasciola hepatica*. Sivas (MO): Sivas Cumhuriyet Univ; 2020.