

Original Article

Biochemical Evaluation of Antioxidant Enzyme Activities and Lipid Peroxidation Level Associated with Liver Enzymes in Patients with Fascioliasis

Kamil Jabbar, D¹*

1. College of Engineering, University of AL-Qadisiyah, Al Diwaniyah, Qadisiyyah Province, Iraq

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Corresponding Author: durat.kamil@qu.edu.iq

Abstract

Fascioliasis, which is caused by infection with *Fasciola gigantica* and *Fasciola hepatica*, is a zoonotic disease with a global distribution. This comparative study aimed to investigate antioxidant enzyme activities and oxidative status of chronic fascioliasis patients. In this study, 20 patients were compared with 10 controls and the levels of superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GPX), catalase (CAT), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes were evaluated. The results showed that MDA, CAT, AST, and ALT levels were higher in patients than in controls, while SOD and GPX levels were higher in controls than in patients ($P \leq 0.05$). Moreover, the enzyme MDA showed a positive correlation with SOD and GPX in the infected group. The enzyme SOD had an indirect correlation with CAT and a direct correlation with GPX. The positive correlation between ALT and AST was shown to be extremely significant ($P \leq 0.05$). The significant decrease in antioxidant enzymes and an increase in serum lipid peroxidation in the red blood cells of patients with fascioliasis indicated the presence of oxidative stress, which showed inflammation and oxidative stress, the pathogenesis of which was indicative of the stage of infection.

Keywords: Fascioliasis, Liver enzymes, Oxidative state, Reactive oxygen species

1. Introduction

The liver trematode parasites *Fasciola gigantica* and *Fasciola hepatica* are food- and water-borne parasitic zoonosis causing Fascioliasis and affecting both grazing animals and humans (1). The global prevalence of this human illness is estimated to be 2.4-17 million people (2). WHO (3) considers this illness a serious infection; therefore, it has been regarded as the main concept for chemotherapy. This disease has become a significant clinical and epidemiological health concern in several countries due to the availability of a particular snail intermediate host and a broad variety of reservoir hosts that continue to be an important source of infection (4). The patient in the chronic stage has

gastrointestinal problems. The mature parasite may be discovered when it leads to hepatic malfunction by causing obstacles in the bile ducts; therefore, these symptoms are often really complex that they are identified in surgery. Serological, parasitological, histopathological, and radiological tests are all important diagnostic techniques (5).

The immigration of larva of *Fasciola* spp. into the host's liver causes an inflammatory response, which is followed by fibrosis and cirrhosis (6, 7). Lipid peroxidation in cells and tissues is an excellent indication of oxidative stress. The polyunsaturated fatty acid-derived lipid peroxides are not stable and break down to create some chemicals, which include

malondialdehyde (MDA) that may be a useful reliable marker for lipid peroxidation (8). Both lipid peroxidation (LPO) and reactive oxygen species (ROS) are produced in hemorrhagic shock, parasite infection, and liver surgery. The production of ROS is considered part of the regular metabolism of the cell. Overproduction of ROS, such as hydroxyl radicals, superoxide anions, and hydrogen peroxide, in such activities as gene translation, mitogenic signal transduction, and cell proliferation control, act as intracellular messengers. (7). Overproduction of ROS *in vivo* may impair cell function (9, 10). When the antioxidant systems are disrupted or ROS inhibition is ineffective, ROS attack causes alterations in molecular structure and changes in biological characteristics that can be detrimental to the cell (11). Major protein subfamilies should be released from the liver flukes *in vitro*, and these proteins include enzymes with important antioxidant functions (9, 10). These enzymes include superoxide dismutase (SOD) which allows the superoxide radical to be broken down into H₂O₂ and H₂O. The SOD enzyme is highly important in defending against ROS; moreover, it is extremely functional in breaking the superoxide radical to remove the toxicity (7).

This study aimed to investigate the biochemical evaluation of antioxidant enzyme activities and lipid peroxidation levels associated with liver enzymes among people suffering from chronic fascioliasis as measured by monitoring the status and activity of each enzyme of catalase (CAT), SOD, MDA, and GPX, and also estimate the levels of the liver enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in *Fasciola* spp. infection.

2. Materials and Methods

2.1. Study Design and Sampling

In this study, samples were evaluated in two groups, including group I (n=20) consisting of individuals with chronic fascioliasis who were negative for other parasite diseases based on stool examination, and group II (n=10) involving healthy individuals (control). All

patients showed symptoms of fasciosis for 4 months and had egg excretion in fecal tests. A serological test was performed to confirm the control group, and seronegative samples were used. The study excluded all the individuals who already were suffering from any type of liver disease or any kind of liver viral infection or had previously been infected with any other parasite disease that affected the liver or its normal function. Moreover, the study excluded any patient individuals who had renal problems, diabetic disease, cardiac disease; patients who were taking any fat lowering medication or treatment; and the individuals who were receiving antioxidant treatments for at least the preceding 6 months. Blood samples were collected and added into two types of tubes, namely anticoagulated tubes containing sodium ethylenediaminetetraacetic acid and normal tubes lacking anticoagulant.

2.2. Measurement of Oxidative Stress Parameters

An amount of 1 mL of anticoagulant blood was collected for the process of hematologic analysis, and hemoglobin levels were measured using the automatic blood counter. Plasma and erythrocytes were isolated from the residual anticoagulated blood. Plasma was isolated and erythrocyte lysate was produced by centrifugation at 8,000g for 15 min. For erythrocyte lysis, 0.5 mL of the cell suspension was dissolved in 2 mL of cold water after thrice washing the erythrocyte mass with a physiological solution. Subsequently, 2 mL of water was added and then 0.2 mL ethanol/chloroform (3:5/v:v) was added to 0.2 mL of lysate to precipitate hemoglobin. Afterward, the tubes were shaken for about 5 min before the centrifugation at 8,000g for about 20 min. In order to evaluate enzymatic activity, samples were kept at -20°C for fewer than 3 months (12). The enzyme activities were determined using the supernatant.

2.3. Measurement of Antioxidant Enzymes Activities

Superoxide dismutase activity can be measured using the xanthine-xanthine oxidase system, which is considered a superoxide generator. The activity of the enzyme SOD (EC 1.15.1.1) was measured (Cu, Zn, and

Mn). Initially, 1 mL of ethanol/chloroform mixture (5/3, v/v) was added to a similar amount of material, followed by the centrifugation at 4,000g and the measurement of the activity of the enzyme in the supernatant ethanol phase (Kaya, 2007). The activity was reported by the units per gram of the hemoglobin, and the SOD unit was the quantity of the enzyme that inhibited the nitroblue tetrazolium reduction rate by 50%.

The next step was monitoring the activity of the enzyme GPX. Therefore, GPX (EC 1.6.4.2) activity was monitored and the reaction of the enzyme in the tube, which also included reduced glutathione, sodium azide, glutathione reductase, and nicotinamide adenine dinucleotide phosphate, was started using hydrogen peroxide, and then any difference in the absorbance at 340 nm was measured by the spectrophotometer. In erythrocyte samples, the activity was assessed by the units/gram of hemoglobin (13).

The CAT activity was measured by the rate constant of H₂O₂ breakdown at 240 nm. The results were given in k (s⁻¹) per gram of the hemoglobin. The tests were carried out at an ambient temperature of 25°C (14).

2.4. Measurement of Lipid Peroxidation

Malondialdehyde was measured in serum samples right away. The thiobarbituric acid reaction technique was used to determine MDA levels in blood samples. An amount of 2 mL of blood samples without using anticoagulant was then centrifuged at 8000g for about 10 min at 4°C to obtain the serum. At 532 nm, the absorption of thiobarbituric acid reactive compounds was compared with the reference curve line for the MDA equivalents which was produced by the acid-catalyzed hydrolysis of 1,1,3,3-Tetramethoxypropane. Concentrations of the MDA were measured by the units of micromoles/liter (14).

2.5. Measurement of Liver Enzymes

The levels of serum ALT and AST activity were assessed using diagnostic kits (Biomerieux) based on the instructions provided by the manufacturer.

2.6. Statistical Analysis

The collected data were analyzed in SPSS software (version 15; SPSS Inc, Chicago, IL, USA) using a student t-test to evaluate the statistical significance of the difference between the two research groups. Moreover, the relationship between continuous variables was determined by the Pearson correlation coefficient, and the sensitivity and specificity of markers were assessed by the receiver operating characteristic (ROC) curve. A p-value of < 0.05 was considered significant.

3. Results

The studied enzymes, namely MDA, CAT, SOD, GPX, and AST, showed a very detectable variation in the results between the control and infected groups. It was revealed that MDA, AST, ALT, and CAT levels were higher in the infected group with *Fasciola* spp. than in the controls; however, SOD and GPX levels were higher in the controls than in the infected group ($P \leq 0.05$) (Table 1). A significant negative correlation was observed between the enzyme MDA and both CAT and AST. Furthermore, the enzyme MDA showed a strong positive correlation with both SOD and GPX in the infected group. The enzyme SOD had an indirect correlation with CAT and a direct correlation with GPX. The positive correlation between ALT and AST was reported to be extremely significant ($P \leq 0.05$) (Figure 1). The ROC curve revealed that the levels of MDA, CAT, AST, SOD, and GPX were utilized to diagnose infection with *Fasciola* spp. In this regard, MDA levels of 0.495 could be utilized in diagnosis with a 100% specificity, whereas CAT levels of 24.75 may be employed with a sensitivity and specificity of about 82% and 99%, respectively. Superoxide dismutase of 1,259.25 had a sensitivity and specificity of 100% and 89%, respectively, and GPX of 41.5 had a sensitivity and specificity of 100% and 99%, respectively (Table 2; Figures 2 and 3).

Table 1. Levels of enzymes in patients with chronic fascioliasis disease and controls

Parameter	Group 1 cases (n=20) Mean±SD	Group 2 controls (n=10) Mean±SD	t	P-value
AST (U/L)	53.14±19.92	27.02±9.01	5.11	0.0001**
GPX (U/gHb)	13.007±1.09	75.88±6.11	34.076	0.0001**
ALT (U/L)	40.14±18.19	28.81±7.40	2.418	0.023*
SOD (U/gHb)	975.76±190.03	1,401.49±70.02	-8.028	0.0001**
CAT (K/gHb)	30.004±2.99	21.03±2.01	7.055	0.0001**
MDA (Lmol/L)	0.98±0.19	0.19±0.03	16.988	0.0001**

MDA: Malondialdehyde; GPX: Glutathione peroxidase; ALT: Alanine aminotransferase; SOD: Superoxide dismutase; CAT: Catalase; AST: Aspartate aminotransferase

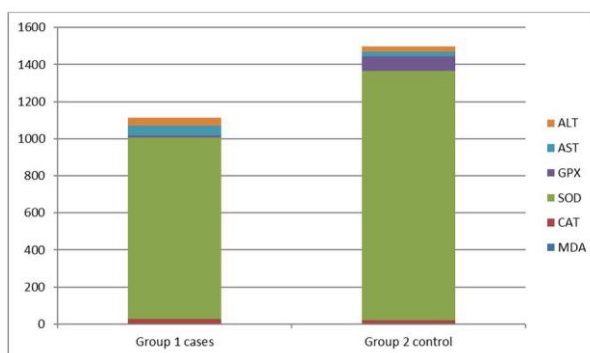


Figure 1. Correlation between enzyme levels in individuals with chronic fascioliasis and controls

Table 2. Presents tested markers and calculated parameters resulting from the analysis the ROC curve were used for distinguishing and comparing the controls with the infected group

Enzyme	AUC	CI	P	Sensitivity (%)	Specificity (%)	P-value
SOD (+ve if $\leq 1,259.25$)	1.004	1.001	0.99	100	89	0.0001**
GPX (+ve if ≤ 41.5)	0.98	0.99	0.99	100	99	0.0001**
ALT (+ve if ≥ 29)	0.699	0.501	0.890	73	59	0.081
CAT (+ve if ≥ 24.75)	0.899	0.879	0.98	82	99	0.0001**
AST (+ve if ≥ 36)	0.901	0.802	0.98	82	79	0.001**
MDA (+ve if ≥ 0.495)	1.000	1.000	1.0	100	100	0.0001**

AUC: Area under the curve; CI: Confidence interval; P: Probability; MDA: Malondialdehyde; GPX: Glutathione peroxidase; ALT: Alanine aminotransferase; SOD: Superoxide dismutase; CAT: Catalase; AST: Aspartate aminotransferase

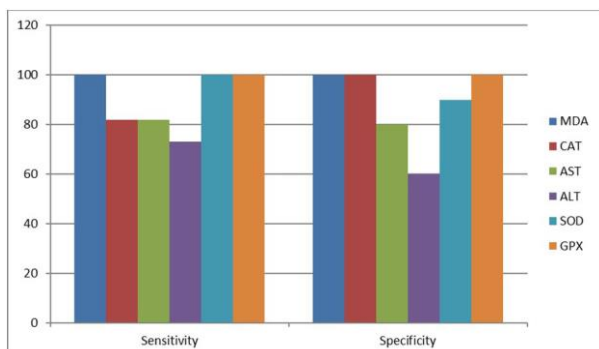


Figure 2. Correlation between sensitivity and specificity of enzymes

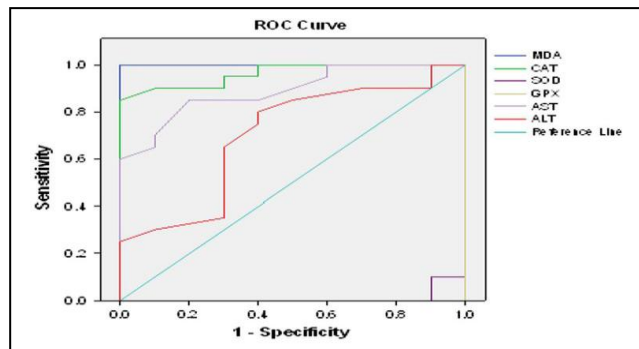


Figure 3. Comparative analysis between enzymes (ROC curve)

4. Discussion

Fasciola hepatica and *F. gigantica* are liver fluke trematodes in ruminants and humans, and infection occurs through encysted metacercariae. In the life cycle of the parasite, migration is seen in the liver parenchyma tissue, which leads to inflammation, stimulation of the immune system, and changes in enzymes (2). The results of studies have shown that inflammatory reactions lead to changes in erythrocyte antioxidant enzyme activities of patients with fascioliasis (4).

Gradual reduction of the molecular O₂, which is a critical component of proper cellular activity, leads to the production of free radicals (14). With a malfunction in the oxidation/antioxidants system, however, excessive production and inadequate clearance of free radicals cause permanent cell damage (15, 16). Numerous parasitic infections have been linked to ROS, such as *Leishmania* (17, 18). As a result, its inhibition causes a rise in peroxide buildup to hazardous levels (19). Our findings, which indicated decreased GPX activity compared to the control group, confirmed that oxidative stress persisted in the fascioliasis chronic phase ($P \leq 0.05$).

The enzyme SOD had been discovered in a variety of parasitic worms, including *Dirofilaria immitis*, *Brugia pahangi*, and *Schistosoma mansoni* (19, 20). Infection with *F. hepatica* was associated with a high amount of superoxide radicals, suggesting that they might act as a precursor to other ROSs (9, 21). In our study, the activities of the antioxidant enzyme of the erythrocyte for SOD in patients with chronic *Fascioliasis gigantica* were lower, compared to the controls. This finding was consistent with the results of previous studies by Kolodziejczyk, Siemieniuk (7) and Gregorevic, Lynch (10) reporting that SOD activity was reduced, resulting in higher superoxide anions and a reduction in the activities of the major antioxidant enzymes (7, 9). The enhanced activity of CAT was the sole exception in this research. The increase in CAT activity might be explained by oxidants produced in the liver activating

the expression of the enzyme gene (7, 11). Regarding the enzymes CAT, SOD, and GPX, obvious differences were highly recognizable among the controls and the cases under the study. It was also found that the enzyme SOD was associated with CAT and GPX ($P \leq 0.05$). According to the results of some research (22-24), the enzyme CAT was a significant intracellular antioxidant. However, Finzi, Chiavegatto (25) observed that CAT activity was irrelevant with fascioliasis because no significant difference was found between the controls and the individuals with fascioliasis, even small differences.

Although LPO is a continuous physiological process, numerous findings demonstrate that peroxidation has an essential association with the development of several parasite illnesses (12, 23). Reactive oxygen species-induced LPO causes the disorganization and eventual rupture of cell membranes, resulting in necrotic death (26). In human cells, MDA, one of the end products of LPO, increases when ROS rises (16). Serum MDA levels of patients in this study were higher than those in the control group ($P \leq 0.05$); therefore, this increase might be a sign of cell damage to the liver caused by *F. gigantica*. (27). The experimental result indicated that a reduction in the mechanism of the defense activity, which protects the body against the harmful effects of free radicals, might be a primary cause for elevated levels of the enzyme MDA in the individuals who were infected with *Fasciola hepatica* (27). A strong correlation was observed between CAT and MDA among the patients in our research, while MDA had a highly significant negative correlation with both SOD and GPX. Kaya, Sutcu (26) examined the association of the activity of the enzymes GPX, MDA, CAT, and SOD with the infection with *F. hepatica*. It seems that oxidative stress could be one of the fundamental causes leading to the development of this illness.

Authors' Contribution

Study concept and design: D. K. J.

Acquisition of data: D. K. J.

Analysis and interpretation of data: D. K. J.
 Drafting of the manuscript: D. K. J.
 Critical revision of the manuscript for important intellectual content: D. K. J.
 Statistical analysis: D. K. J.
 Administrative, technical, and material support: D. K. J.

Ethics

This study was approved by the Ethics Committee of the University of Al-Qadisiyah, Iraq. Informed consent was obtained from all participants.

Conflict of Interest

The authors declare that they have no conflict of interest.

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