Lipid peroxidation level and antioxidant enzyme activities in the blood of patients with acute and chronic fascioliasis

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Summary

Objective: In this study, we investigated the relationship between fascioliasis and serum malondialdehyde (MDA) levels, superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) activities. We also investigated whether there are significant differences in MDA levels and antioxidant enzymatic activities between acute and chronic fascioliasis.

Methods: Forty fascioliasis patients who were diagnosed by ES-ELISA positivity were included in this study. The patients were classified as 18 with acute and 22 with chronic fascioliasis.

Results: In patients with fascioliasis, levels of MDA were statistically higher and erythrocyte SOD and GPx activities were statistically lower than in healthy controls. MDA levels were found to be higher in patients with acute fascioliasis than in patients with chronic fascioliasis although MDA levels were significantly higher in patients with chronic fascioliasis than in controls. There was no statistically significant difference between the two groups for the antioxidant enzyme activities.

Conclusion: The results of this study may indicate that fascioliasis produces specific effects on the antioxidant defense mechanisms due to its inflammatory character. Our results also allow us to suggest that oxidative stress has an important role in the pathogenesis of fascioliasis and the persistence of this oxidative stress can be one of the underlying factors in the pathogenesis of the chronic disease.

Introduction

Fascioliasis is an infection of the bile ducts caused by Fasciola hepatica, the liver fluke of sheep, cattle, and man.1 Fascioliasis is an important helminthic disease with an estimated two million cases worldwide, and its incidence has apparently increased since 1980.2 In our geographic region in
Turkey, recent prevalence rates of fascioliasis were 6.1% and 14.5% in patients with eosinophilia and chronic urticaria, respectively.\textsuperscript{3,4} Fascioliasis can be classified as an acute or chronic disease. The acute phase (parenchymal stage) describes the fluke migration into the bile duct where parasites digest hepatic tissue and cause extensive parenchymal destruction and immunologic and inflammatory reactions. The chronic phase develops months after initial infection and consists of inflammation and hyperplasia of the epithelium caused by adult flukes residing in the bile ducts.\textsuperscript{5} In addition to the mechanical effects on the bile ducts, excretory–secretory (E–S) products such as proteases may contribute to the tissue damage seen in fascioliasis.\textsuperscript{6} In addition, there is evidence that infected tissue is under oxidative stress during the parenchymal stage of the infection.\textsuperscript{7,8}

Normal cellular metabolism involves the production of reactive oxygen species (ROS).\textsuperscript{9} Low levels of ROS are vital for proper cell functioning, while excessive in vivo generation of these products can adversely affect cell functioning.\textsuperscript{10,11} Production of ROS and lipid peroxidation (LPO) occurs in clinical settings such as hepatic surgery, hemorrhagic shock, and parasitic infections.\textsuperscript{12,13} Malondialdehyde (MDA) is one of the final products of LPO in human cells, and an increase in ROS causes overproduction of MDA. Accordingly, the MDA level is considered a surrogate marker of oxidative stress.\textsuperscript{14,15} The major intracellular antioxidant enzyme, superoxide dismutase (SOD), specifically converts superoxide radicals to hydrogen peroxide,\textsuperscript{16} and catalase and glutathione peroxidase (GPx) detoxify hydrogen peroxide to water.\textsuperscript{17} Reactive oxygen species including superoxide anion, hydrogen peroxide, and hydroxyl radical act as subcellular messengers in complex processes such as mitogenic signal transduction, gene expression, and regulation of cell proliferation when they are generated excessively or when enzymatic and non-enzymatic defense systems are impaired.\textsuperscript{18}

There are a few studies on the association between chronic fascioliasis and oxidative stress,\textsuperscript{19–21} but there is a paucity of data on the blood antioxidant enzyme activities of patients with acute or chronic fascioliasis. In addition, elucidation of precise pathogenic mechanisms may have particular relevance for the treatment of fascioliasis. The aim of this study was to measure the serum lipid peroxidation level and SOD, GPx, and catalase activities in erythrocytes, and to compare the results with those of healthy controls.

Patients and methods

Patients

Forty patients with fascioliasis were included in this case-control study. For each subject the diagnosis of fascioliasis was established serologically using a modified enzyme-linked immunosorbent assay (ELISA) prepared with ES antigens in our laboratory or by finding eggs of Fasciola in stools, and clinical and laboratory parameters were subsequently evaluated. There were 18 patients with acute fascioliasis (10 male, 8 female; mean age 45 years) and 22 patients with chronic fascioliasis (11 male, 11 female; mean age 47 years). There were no ethnic differences between patients; nine subjects were from urban and 31 were from rural areas. Besides duration of the disease, patients were classified as having acute or chronic fascioliasis according to clinical, laboratory (liver enzymes, eosinophilia, eggs in stools), and radiologic findings. Subjects with symptoms <4 months were considered as having acute infection, and patients with symptoms for >4 months were deemed to have chronic infection. A control group consisted of 40 healthy individuals (20 males, 20 females; mean age 41 years) who were seronegative by ELISA assay for fascioliasis, as well as seronegative for hepatitis B and C viruses.

Patients with liver dysfunction, diabetes mellitus, cardiac or renal failure, and those taking antioxidant or lipid-lowering therapy within the previous six months were excluded from the study. This study was approved by the Medical Faculty Ethics Committee of Suleyman Demirel University, and written informed consent was obtained from all study subjects and controls.

Blood collection and preparation of blood samples

After an overnight fast, venous blood (10 mL) was taken from an antecubital vein using a monovette blood collection system, inoculated into both non-anticoagulated and anticoagulated tubes (containing sodium EDTA), and protected from light. Serum was obtained from 2 mL of blood without anticoagulant. One mL of anticoagulated blood was used for hematologic analysis. The remaining anticoagulated blood was separated into plasma and erythrocytes by centrifugation at 1500 × g for 10 min at +4 °C. The erythrocyte samples were washed three times in cold isotonic saline (0.9%, v/w) and then hemolyzed with a nine-fold volume of phosphate buffer (50 mM, pH 7.4). After addition of butylhydroxytoluol (4 μL per mL), hemolyzed erythrocyte samples were stored at −30 °C for <3 months pending measurement of enzymatic activity. Serum samples were used for immediate lipid peroxidation and all hematological parameters were measured within six hours of venipuncture.

Determination of lipid peroxidation levels

LPO (as malondialdehyde, MDA) levels in serum samples were measured using the thiobarbituric acid reaction method of Draper and Hadley.\textsuperscript{15} Quantification of thiobarbituric acid reactive substances was determined at 532 nm by comparing the absorption to the standard curve of MDA equivalents generated by acid-catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane. Values of MDA were expressed as nmol/mL.

Antioxidant enzyme assay

SOD activity determination: total (Cu–Zn and Mn) SOD (EC 1.15.1.1) activity was determined according to the method of Woolliams et al.\textsuperscript{22} The test is based on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the supernatant after 1.0 mL ethanol/chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged at 4000 × g. One
Lipid peroxidation and antioxidant enzyme in fascioliasis

A unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. SOD activity was also expressed as units per gram of hemoglobin in erythrocyte samples.

Glutathione peroxidase activity determination: GPx (EC 1.6.4.2) activity was measured by the method of Paglia and Valentine. The enzyme reaction in the tube, which contains NADPH, reduced glutathione, sodium azide, and glutathione reductase, was initiated by addition of hydrogen peroxide, and the change in absorbance at 340 nm was monitored by a spectrophotometer. Activity was given as units per gram of hemoglobin in erythrocyte samples and all samples were assayed in duplicate.

Catalase activity determination: catalase (EC 1.11.1.6) activity was determined according to Aebi’s method. The test is based on the determination of the rate constant (k) or the H₂O₂ decomposition rate at 240 nm. Results were expressed as k (s⁻¹) per gram hemoglobin. Assays were performed at room temperature (25°C).

Hemoglobin determinations: Hemoglobin values were determined using an automated blood counter (Beckman Coulter, Miami, USA).

Statistical analysis

All results are expressed as means with 95% confidence intervals (CI). Statistical significance was evaluated using Student’s t, Chi-square, and one-way Anova tests and SPSS (version 10.0; SPSS, USA) software package. A p value <0.05 was considered to be statistically significant.

Results

Demographic properties and means (95% CI) of MDA, SOD, catalase, and GPx levels in fascioliasis patients and controls are shown in Table 1. In patients with fascioliasis, levels of MDA were higher and erythrocyte SOD and GPx activities were lower than in controls, and these differences were statistically significant (p < 0.05). While the catalase activity was higher in fascioliasis patients than in controls, the difference was not statistically significant.

Demographic features and clinical and laboratory findings of acute and chronic fascioliasis patients are shown in Table 2. The most frequent symptoms in acute fascioliasis patients were abdominal pain, fever, weight loss, and allergic reactions, and in patients with chronic fascioliasis they were abdominal pain, pruritus, and urticaria. Eosinophilia was detected in only three chronic fascioliasis patients but in all patients with acute fascioliasis. Furthermore, ALT and ALP levels were elevated in 13 and 12 acute fascioliasis patients, respectively, and GGT and ALP levels were elevated in six and five of the chronic fascioliasis patients, respectively. In addition, F. hepatica eggs were detected in four chronic fascioliasis patients.

Table 1  Demographic features, serum MDA levels, and erythrocyte enzyme activities in fascioliasis patients and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fascioliasis (n = 40)</th>
<th>Controls (n = 40)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>21/19</td>
<td>20/20</td>
<td>&gt;0.05 (^a)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>46 (41.7–50.2)</td>
<td>40.55 (37.2–43.8)</td>
<td>&gt;0.05 (^b)</td>
</tr>
<tr>
<td>MDA (nmol/mL)</td>
<td>1.42 (1.28–1.56)</td>
<td>0.20 (0.18–0.22)</td>
<td>&lt;0.001 (^b)</td>
</tr>
<tr>
<td>SOD (U/gHb)</td>
<td>1025.61 (861–1189)</td>
<td>1334.54 (1246–1422)</td>
<td>0.001 (^b)</td>
</tr>
<tr>
<td>GPx (U/gHb)</td>
<td>13.61 (12.5–14.6)</td>
<td>77.12 (69.3–84.8)</td>
<td>&lt;0.001 (^b)</td>
</tr>
<tr>
<td>Catalase (k/gHb)</td>
<td>24.89 (21.4–28.3)</td>
<td>21.31 (19–23.6)</td>
<td>&gt;0.05 (^b)</td>
</tr>
</tbody>
</table>

MDA, malondialdehyde; SOD, superoxide dismutase; GPx, glutathione peroxidase. Values expressed as means with 95% confidence intervals.

\(^a\) Chi-square test.

\(^b\) Student’s t-test.

Table 2  Demographic features and clinical and laboratory findings in acute and chronic fascioliasis

<table>
<thead>
<tr>
<th>Feature</th>
<th>Acute fascioliasis (No. of patients/total)</th>
<th>Chronic fascioliasis (No. of patients/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>(10/8)</td>
<td>(11/11)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.8 ± 12.6</td>
<td>46.9 ± 14.2</td>
</tr>
<tr>
<td>Urban/rural</td>
<td>4/14</td>
<td>(5/17)</td>
</tr>
<tr>
<td>Common symptoms</td>
<td>Abdominal pain (16/18)</td>
<td>Abdominal pain (15/22)</td>
</tr>
<tr>
<td></td>
<td>Fever (9/18)</td>
<td>Pruritis (7/22)</td>
</tr>
<tr>
<td></td>
<td>Weight loss (6/18)</td>
<td>Asymptomatic (3/22)</td>
</tr>
<tr>
<td>Radiologic findings</td>
<td>Tunnel-like, branching, hypodense lesions; local and cystic lesions in the liver; parenchymal inflammation</td>
<td>Thickened wall of gallbladder; dilated common bile duct; mass in gallbladder</td>
</tr>
<tr>
<td>Eosinophilia</td>
<td>(18/18)</td>
<td>(3/22)</td>
</tr>
<tr>
<td>High ALT—AST</td>
<td>(13/18–8/18)</td>
<td>(2/22–2/22)</td>
</tr>
<tr>
<td>High ALP—GGT</td>
<td>(12/18–7/18)</td>
<td>(5/22–6/22)</td>
</tr>
<tr>
<td>Eggs in stools</td>
<td>(0/18)</td>
<td>(4/22)</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyltransferase.
Serum MDA, erythrocyte SOD, GPx, and catalase activities in acute and chronic fascioliasis patients are shown in Table 3. According to the duration of infection, MDA levels were significantly higher in patients with acute fascioliasis compared to patients with chronic fascioliasis \((p < 0.001)\), although MDA levels were significantly higher in patients with chronic fascioliasis compared to controls \((p < 0.01)\), likely indicating the persistence of oxidative stress in the chronic phase. There was no statistically significant difference between the two groups for the antioxidant enzyme activities. However, using the one-way Anova test post hoc, SOD and GPx activities were significantly lower in patients with acute and chronic fascioliasis compared to controls \((p < 0.05)\) and 

<table>
<thead>
<tr>
<th>Level</th>
<th>Acute fascioliasis ((n = 18))</th>
<th>Chronic fascioliasis ((n = 22))</th>
<th>Controls ((n = 40))</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/mL)</td>
<td>1.61 ((1.36—1.86))</td>
<td>1.26 ((1.13—1.39)^a)</td>
<td>0.20 ((0.18—0.22)^b)</td>
</tr>
<tr>
<td>SOD (U/gHb)</td>
<td>1028.02 ((762—1293))</td>
<td>1023.62 ((798—1248)^c)</td>
<td>1334.54 ((1246—1422)^d)</td>
</tr>
<tr>
<td>GPx (U/gHb)</td>
<td>14.34 ((13.1—15.5))</td>
<td>13.01 ((11.3—14.6)^c)</td>
<td>77.12 ((69.3—84.8)^b)</td>
</tr>
<tr>
<td>Catalase (k/gHb)</td>
<td>23.29 ((18.1—28.4))</td>
<td>26.19 ((21.1—31.2)^c)</td>
<td>21.31 ((19—23.6)^e)</td>
</tr>
</tbody>
</table>

MDA, malondialdehyde; SOD, superoxide dismutase; GPx, glutathione peroxidase. Values expressed as means with 95% confidence intervals.

\(p < 0.001\) with acute fascioliasis.

\(p < 0.001\) with both acute and chronic fascioliasis.

\(p > 0.05\) with acute fascioliasis.

\(p < 0.05\) with both acute and chronic fascioliasis.

\(p > 0.05\) with both acute and chronic fascioliasis.

Discussion

Inflammatory reactions lead to a decrease in erythrocyte antioxidant enzyme activities of patients with fascioliasis and other parasitic infections, while MDA levels in serum or plasma increase in the presence of inflammation. In the present study, erythrocyte antioxidant enzyme activities were lower in study subjects compared to controls, while serum MDA levels in patients with fascioliasis were higher than in controls. These results likely reflect the inflammation and oxidative stress that persists in the chronic phase of fascioliasis. Others have similarly reported significant improvement of SOD and GPx activities and in lipid peroxide levels after antioxidant vitamin C and vitamin E supplementation plus triclabendazole compared to values after drug therapy alone. These authors postulated that such findings were due to the protective effects of these vitamins against oxidative damage.

Oxidative cellular injury may occur in the course of fascioliasis as the consequence of tissue destruction produced by toxic secretions of the flukes. In the acute phase of fascioliasis, juvenile flukes cause localized or generalized toxic and immunologic reactions that result in an increase in oxidative stress and mechanical destruction of liver tissue and peritoneum. There is evidence that the infected rat liver is under oxidative stress during the parenchymal stage of infection. In the chronic stage, the fluke protects itself from immunologic host responses by escaping into bile ducts. During this period, inflammatory, necrotic, and fibrotic lesions develop as a result of parasites migrating through the liver towards the bile ducts. Hepatic fibrogenesis is also related to stimulation by lipid peroxidation of collagen synthesis in lipocytes and activation of the release of profibrogenic cytokines by Kupffer cells. In the course of fascioliasis, many E-S antigens not yet fully identified may cause antigenic stimulation in the host. It is also reported that E–S components of F. hepatica may also exert direct immune suppressive effects through the activity of proteinases on immunoglobulin molecules. However, the toxic and immunologic effects of these antigens in the host have yet to be completely clarified.

Both catalase and GPx detoxify hydrogen peroxide to water, although the latter appears to have more potent activity. While GPx activities were lower in our patients with fascioliasis compared to controls, catalase activities were not significantly different between the groups, although the reason for this is unclear.

Chronic asymptomatic fascioliasis may be characterized by an absence of clinical features, parasite eggs in the stool, elevated liver enzymes, eosinophilia, and radiographic findings, and identified only by seropositivity. Asymptomatic patients are often detected during contact screening, and although the precise frequency is not certain, asymptomatic infection may constitute up to 20% of cases of chronic fascioliasis in some series. Our results suggest that biochemical markers of oxidative stress may be diagnostically useful in patients with chronic asymptomatic Fasciola infection.

In conclusion, in patients with fascioliasis the findings of increased serum lipid peroxidation and decreased antioxidant enzymes in erythrocytes indicate the presence of persistent inflammation and oxidative stress. These results support the hypothesis that there is an imbalance between ROS production and antioxidant host defenses in inflammatory fascioliasis disease. We suggest that oxidative stress may be one of the factors underlying the pathogenesis of this chronic parasitic infection.

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Conflict of interest: No conflict of interest to declare.

References


