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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

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Turkey, recent prevalence rates of fascioliasis were 6.1% and 14.5% in patients with eosinophilia and chronic urticaria, respectively.[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.[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.[6] In addition, there is evidence that infected tissue is under oxidative stress during the parenchymal stage of the infection.[7,8]

Normal cellular metabolism involves the production of reactive oxygen species (ROS).[9] Low levels of ROS are vital for proper cell functioning, while excessive in vivo generation of these products can adversely affect cell functioning.[10,11] Production of ROS and lipid peroxidation (LPO) occurs in clinical settings such as hepatic surgery, hemorrhagic shock, and parasitic infections.[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.[14,15] The major intracellular antioxidant enzyme, superoxide dismutase (SOD), specifically converts superoxide radicals to hydrogen peroxide,[16] and catalase and glutathione peroxidase (GPx) detoxify hydrogen peroxide to water.[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.[18]

There are a few studies on the association between chronic fascioliasis and oxidative stress,[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 $\leq$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 $\times$ *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.[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.[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 $\times$ *g*. One