CLINICAL STUDIES

Serum Malondialdehyde Levels as a Biomarker of Cellular Injury In Human Fascioliasis

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Abstract: Macrophages, neutrophils and other phagocytic cells are key components of the antiparasitic, antimicrobial and tumoricidal immune responses. These cells are capable of generating large amounts of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS have a possible role in the pathogenesis of parasitic disease. Lipid peroxidation is a well-established mechanism of cellular injury and is used as an indicator of oxidative stress in cells and tissues. To examine oxidant status and lipid peroxidation in fascioliasis patients, the malondialdehyde (MDA) (an end-product of lipid peroxidation) has been studied. Serum MDA level was measured in 34 patients infected with *Fasciola gigantica* and their age and gender were matched to 36 healthy controls. The difference between MDA levels of patients infected with *Fasciola gigantica* and the control group was statistically significant both in females ($P< 0.05$) and males ($P< 0.05$) with no correlation between age and MDA levels both in females and males of patient and control group. The high infection/control ratio of MDA concentration and the significant correlation strongly indicate the occurrence of oxidative stress and lipid peroxidation as a mechanism of tissue damage in cases of *F. gigantica* infection.

Keywords: Fascioliasis, Reactive Nitrogen Species, Reactive Oxygen Species

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Introduction

Fascioliasis is a world wide, zoonotic disease of herbivores that cause a massive economic loss. It is caused by trematodes of the genus *Fasciola* can cause disease in human. Until 1990, human fascioliasis was considered a secondary disease [1]; however, in the last decade, Fascioliasis is recognized as an important infectious condition by the World Health Organization [2] and the number of reported cases has increased, involving 51 countries worldwide [3]. Today we know that fascioliasis is an important human disease, with up to 17 million people infected worldwide [4]. Human fascioliasis is present in Egypt since ancient times. It is becoming an increasingly important clinical and epidemiological health problem in Egypt, with prevalence of suitable snail intermediate host and the presence of large varieties

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of reservoir hosts that remain as a continuous source of infection [5,6]. *F. hepatica* predominates in temperate zones, while *F. gigantica* is found in most continents, primarily in tropical regions [3]. In Egypt; *Fasciola gigantica* was considered the endogenous species of *Fasciola* found in the Nile Delta, while *F. hepatica* was thought to be present only in imported animals [7-10]. The prevalence of human fascioliasis may be underestimated. Humans become infected after eating aquatic plants containing encysted organisms or by drinking contaminated water. The clinical picture of human fascioliasis in the acute stage is remarkable, with high eosinophilia, hepatomegaly and fever; but in the chronic stage, most cases have vague or non-specific gastrointestinal symptoms [11]. Some of them are complicated and, as a result, are diagnosed in operating rooms, where adult parasites are found obstructing the bile ducts or causing hepatic dysfunction [12-14]. The diagnostic aspects include parasitological, radiological, and histopathological studies, and serological tests. Diagnosis and treatment of fascioliasis is not easy, as physicians rarely encounter this disease, and effective drugs are not available in many countries [14].

Reactive oxygen species (ROS) are formed and degraded by all aerobic organisms leading to either physiological concentrations required for normal cell function, or excessive quantities, the state called oxidative stress. Due to their high reactivity, ROS are potentially toxic, mutagenic, or carcinogenic [15]. Free radicals are involved in several pathological conditions [16] and, at subtoxic concentration; they have recently been shown to play several crucial physiological roles in biological systems [17]. A possible role(s) of the highly reactive oxygen free radicals in the pathogenesis of parasitic infections has been an active area of research in the recent years [18-20]. Polymorphonuclear neutrophils and monocyte ⁄ macrophage cells play an important role in the host defense [21]. These cells are capable of generating large amounts of highly toxic molecules, such as reactive oxygen species (ROS), including superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH), and reactive nitrogen species (RNS), including nitric oxide (NO) [22] . Bacteria, parasites and tumor cells activate macrophages to synthesize large quantities of NO, with NO having cytotoxic effects on these activators [23, 24]. ROS and RNS are capable of degrading numerous biomolecules, including DNA, carbohydrates and proteins [25]. In addition, ROS and RNS can attack the polyunsaturated fatty acids of membrane lipids causing lipid peroxidation and the disorganization of cell structure and function [25]. Lipid peroxidation is a well-established mechanism of cellular injury and is used as an indicator of oxidative stress in cells and tissues [26]. Lipid peroxides derived from polyunsaturated fatty acid are unstable and can decompose to form a complex series of numerous degradation products (lipid peroxides). Among these, reactive carbonyl compound is the most abundant MDA. As such, measurement of MDA is widely used as an indicator of lipid peroxidation [27]. Increased levels of lipid peroxidation products have been associated with a variety of chronic diseases including parasitic infections [28, 29].

In this study, the serum concentration of MDA (an end-product of lipid per oxidation) was measured in humans with *Fasciola gigantica* to establish its relevance in the pathological mechanism of the disease.

Subjects and Methods

Faecal and serum samples were collected from people who attended National Hepatology and Tropical Medicine Research Institute (NHTMRI) Cairo, Egypt suffering from GIT disturbances. None of them were smokers, had any known illnesses, allergic diseases or intake of steroids or medications such as iron for anemia at the time of sampling. Faecal and serum samples for the control group were obtained from healthy people working in different departments of the NHTMRI. Faecal samples were analyzed within 3 hours after collection. Venous blood (5 ml) was drawn and allowed to clot at room temperature for 1 hour then serum was separated, placed in small tubes, and stored at -20 ^{0}C. The detection was based on the recovery and identification of the characteristic eggs of *Fasciola gigantica* [30, 31] . Investigation was performed by stool examination and indirect haemagglutination (IHA) of Fascioliasis, Schistosomiasis, Amoebiasis and Hydatid disease for all patients and controls. Three stool samples were collected on three consecutive days per subject without preservative, for the faecal analysis that were performed by the wet mount and Lugol`s iodine staining, the modified formalin-ethyl acetate technique (MFECT), the Fluke Finder Technique (FFT).

The conventional method for diagnosis of parasitic infections was the MFECT according to Lumbreras, *et al*., [32] , Elkins *et al.* [33] and Maco, *et al*., [30] , in which 2 g of faecal sample was suspended in saline, strained into a 15-ml conical centrifuge tube and centrifuged for 2 min at 2500 rpm. The supernatant was decanted, and approximately 0.5 ml of sedimented faeces was thoroughly mixed with 9-ml 10% Formalin in the tube. 4-ml amount of ethyl acetate was added and the tube was shaken in an inverted position for 30s and then centrifuged for 2 min at 2000 rpm. The resultant four layers were: solvent, a plug of debris, formalin, and sediment. The plug of debris was loosed by ringing with an applicator stick and the top three layers were decanted. Unstained and iodine-stained mounts were prepared and examined systematically.

This technique is used to diagnose *Fasciola* and other large sized eggs [34]. Two grams of faeces were applied on two successive sieve systems (Fluke finder, Moscow, ID) as shown in figure 1. The upper one of 400 microns/pore which permits the passage of eggs and fine particles only followed by a second one of 80 microns / pore; which prevent the large eggs from passing and keeps them on the second sieve surface. The contents over the second sieve were transferred to a small Petri dish using pushed tap water and then one drop of methylene blue was added and examined under microscope to detect *Fasciola* eggs.

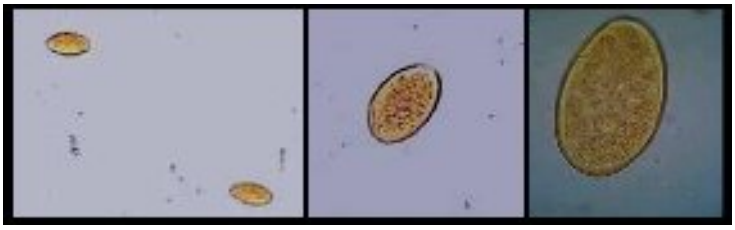

Figure 1: Fasciola gigantica eggs

IHA tests of Fascioliasis, Schistosomiasis, Amoebiasis & hydatid disease was done for all patients and controls sera. IHA test kits were used according to the manufacturer's instructions (IHA, Fumouze Labs, Levallois Perret, France). Serum MDA levels were measured in 34 serum samples of patient only infected with *Fasciola* (Characteristic *Fasciola* egg detected in stool and IHA test for Facioliasis was positive with no mixed infection) and 36 healthy controls. Serum MDA levels were measured for all study subjects by the double heating method [35-37]. The principle of the method was based on the spectrophotometric measurement of the color occurring during the reaction to thiobarbituric acid with MDA. Concentration of thiobarbituric acid reactive substances was calculated by the absorbance coefficient of malondialdehyde-thiobarbituric acid complex and expressed in nmol/ml.

Statistical analysis was performed with SPSS software package (Version 11.0 for Windows). Data were expressed as mean ± standard deviation. For comparison of two groups of continuous variables, independent samples *t*-test was used. A probability value of < 0.05 indicated a statistically significant difference.

Results

The mean age of patients and control group are given in Table 1. The serum level of malondialdeyde is given in Table 2 and Figure 1.

Table 1: Age in patients infected with *Fasciola gigantica* and healthy controls

Characteristics		No	Age (year)
Patients	Females	18	27 ± 16
	Males	16	31 ± 13
Controls	Females	19	32 ± 11
	Males	17	41 ± 10

Table 2: Serum levels of Malondialdehyde (MDA) in patients infected with *Fasciola gigantica* and healthy controls

Characteristics		No	MDA level (nmol/ml)
Patients	Females	18	0.78 ± 0.10
	Males	16	0.74 ± 0.18
Controls	Female	19	0.22 ± 0.11
	Male	17	0.23 ± 0.16

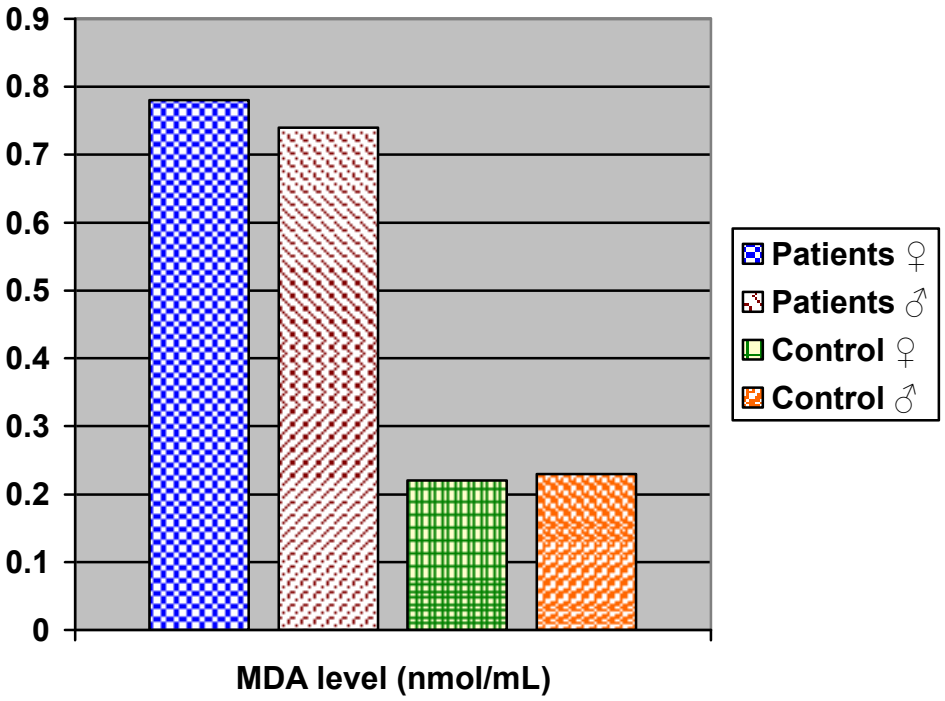

Figure 1: Serum levels of Malondialdehyde (MDA) in patients infected with *Fasciola gigantica* and healthy controls

There is a statistical significance in difference between MDA serum levels of patients and control group both for males ($P< 0.05$) and females ($P< 0.05$) as detailed in table 2. There is no correlation between MDA levels and age ($P< 0.05$) in patient and control groups in both sexes. There is no significant correlation between patient and control group males and females and the MDA levels.

Discussion

Highly reactive oxygen free radicals (ROS) have been indicated in the pathogenesis of various parasitic infections including *Leishmania* [18-20, 38], *Plasmodium falciparum* [39] , *Ascaris lumbricoides* [28] , *Toxoplasma gondii* [40], *Trypanosoma cruzi* [41] and Lipid peroxidation is an ongoing physiological process, but several lines of evidence have suggested an important role for peroxidation in the pathogenesis of several parasitic diseases [42]. Lipid peroxidation caused by ROS results in the disarrangement and ultimately, disruption of cell membranes, which leads to necrotic death. There are several reports indicating that infection with various parasites is associated with a marked elevation in lipid peroxidation [19, 20, 42]. In the present study, increased levels of lipid peroxidation were detected in humans infected with *Fasciola gigantica*. This increase in lipid peroxidation in patients may be considered as an indication of cell injury caused by *Fasciola*. Therefore, it can be suggested that increased levels of MDA in serum of infected animals is related to the host defense against parasitic infection. However, the functional benefit of this process is far from being completely elucidated.

Levels of MDA were remarkably increased in patients infected with *F. gigantica*. The results of this study strongly suggest that one of the main reasons for high MDA levels in patients infected with *F. hepatica* could be decreased activity of the defense system protecting tissues from free radical damage. However, in the patient and control groups, no significant correlation was found between age and MDA levels both in females and males. The results for patients infected with *F. gigantica* could possibly be correlated to high MDA activity in all ages. It is known that lipid peroxidation is a free radical–related process that in biologic systems may occur under enzymatic control or non enzymatically. This latter form is associated mostly with cellular damage as a result of oxidative stress which also involves cellular antioxidants in this process [43]. The high infection/control ratio of MDA concentration and the significant correlation strongly indicate the occurrence of oxidative stress and lipid peroxidation as a mechanism of tissue damage in cases of *F. gigantica* infection.

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