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Evaluation of Serum Malondialdehyde And Nitric Oxide Levels in Patients with Cystic Echinococcosis

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Abstract

The aim of current study was to investigate the difference in the serum Malondialdehyde (MDA) and Nitric oxide (NO) levels between healthy individuals and patients with *Cystic echinococcosis* (CE). CE patients (201 person) and healthy individuals (160 person) as control group were included in this study. Their MDA and NO levels were measured by using spectrophotometric methods. MDA and NO levels were statistically significantly higher in the CE patients group than the healthy group ($p < 0.05$). The results indicate that oxidative stress may have increased in CE patients as a consequence of immune response of the host. Considering results of this study, new approaches that related antioxidant therapies can be developing with further studies in the diagnosis, control and treatment of CE disease.

1. INTRODUCTION

Cystic echinococcosis caused by *Echinococcus granulosus* is a parasitic zoonosis whose incidence ranges from 1 to 150/100 000, especially in Mediterranean coastal countries including especially South America, Africa, the Middle East and Turkey where animal husbandry is common [1, 2]. Liquid-filled, cyst-shaped larvae of *E. granulosus* which is the cause of disease, can be found in many organs especially in the liver in intermediate host pets such as dogs, cattle, sheep, goats, and in human beings [1].

In parasitic infections, immune system of the host protects it against parasite through various cellular mechanisms. Reactive oxygen and nitrogen derivatives produced by activated phagocytes play a critical role in cellular protection mechanisms as cytotoxic agents [3, 4]. Invading microorganisms that have been phagocytized by phagocytic cells such as neutrophils are destroyed by a series of chemical reactions called "respiratory burst". Therefore, respiratory burst has a central role in the immunity of the host. However, these cytotoxic agents also cause oxidative tissue damage [5]. Previous studies have shown that the amounts of free radicals in host cells infected with different parasite species increased and due to this increase, oxidative damage in cells and tissues occurred [6-10]. Lipid peroxidation initiates by removing a hydrogen atom from the unsaturated fatty acid chain included in membrane structure as a result of excess production of reactive oxygen derivatives. Thereupon, fatty acid chain gains the qualification of lipid radical. Lipid peroxidation is a very damaging chain reaction and damages membrane structure directly and other cell components indirectly by producing reactive aldehydes. Malondialdehyde (MDA), occurred as a result of lipid peroxidation, is considered as an indicative of oxidative damage in tissue and cells [11].

Nitric oxide (NO) is an important mediator having free radical properties and playing a critical role in many physiological and pathophysiological processes. It is, biosynthesized endogenously from L-arginine, oxygen, and NADPH by various nitric oxide synthase (NOS) enzymes in vivo. NOS has three

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isoforms: endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). The first two forms are constitutive and are responsible for the production of endothelial NO as calcium dependent; iNOS is independent from calcium and responsible for the synthesis of high amounts NO from macrophages by stimulation with NADPH oxidase activation [12]. NO in high concentration produced by macrophages are effective in cytostatic and cytotoxic processes due to their radical nature. Nitric oxide produced by iNOS is the non-specific defense mechanism of the host and has antimicrobial effect [13]. NO, which can reach milimolar concentrations as a result of increasing in iNOS expression and activity, cannot maintain its stability in the presence of oxygen and forms reactive nitrogen oxide products (NOx) which are stable end products such as nitrite (NO_2^-) and nitrate (NO_3^-) by oxidizing [14]. It has been reported in some studies that NO production has increased in infections caused by metastestod class parasites such as *Schistosoma*, *Fasciola* and *Echinococcus* and that NO may have antiparasitic effects [15-19].

In this study, we attempted to evaluate serum MDA levels, an indicative of lipid peroxidation, and changes in serum NOx levels which play an important role in the antiparasitic immune response and the relationship between these two in order to determine the degree of tissue damage caused by free radical formation in CE patients.

2. MATERIALS AND METHODS

Patients (201 person) who received pre-diagnosis of Cystic Echinococcosis in Refik Saydam Hıfzısıhha and Etlik SSK Hospital between 2003-2005, and whose anti-*E. granulosus* IgG antibodies were found positive by serological techniques (ELISA, IHA) were included in the study. It is taken into consideration that the individuals included in the patients working group do not have any chronic and systemic diseases according to their laboratory and clinical information. The control group in the study was established from those 160 people applied to Etlik SSK Hospital for control and did not have any chronic or systemic disease.

NOx levels have been determined spectrophotometrically by Griess method as total nitrite after reduction of nitrate to nitrite by VaCl_3 [20, 21]. The MDA assay was performed by measuring the thiobarbituric acid and pink complex formed by MDA by spectrophotometer [22]. The studies were carried out at Gazi University Faculty of Science Physiology Research Laboratory.

All values are expressed as arithmetic mean \pm standard error. Results were statistically compared with ANOVA and Mann Whitney U tests according to whether the distribution was homogenous or not; $p < 0.05$ was considered significant.

3. RESULTS

NOx and MDA changes of control group and CE patient groups are shown in *Figure 1*, *Figure 2*. As shown in *Figure 1*, the NOx level in the control group was $49.45 \pm 8.41 \mu\text{mol/L}$ while it increased almost doubling in the patient group and reached to $84.54 \pm 9.72 \mu\text{mol/L}$. The remarkable increase here was statistically significant ($p < 0.05$)

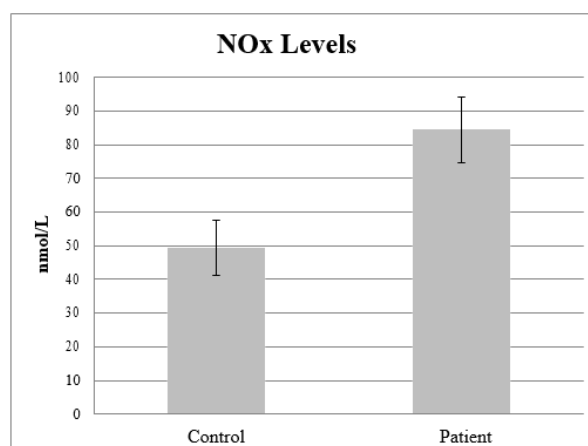


Figure 1. NOx levels change according to the groups (* $p < 0.05$ in comparison to the control group)

The MDA level, indicator of lipid peroxidation, was found to be 4.6 ± 1.4 nmol / L in the patient group and it showed a significant increase ($p < 0.05$) compared to the MDA level of 3.6 ± 0.8 nmol / L in the control group (Figure 2).

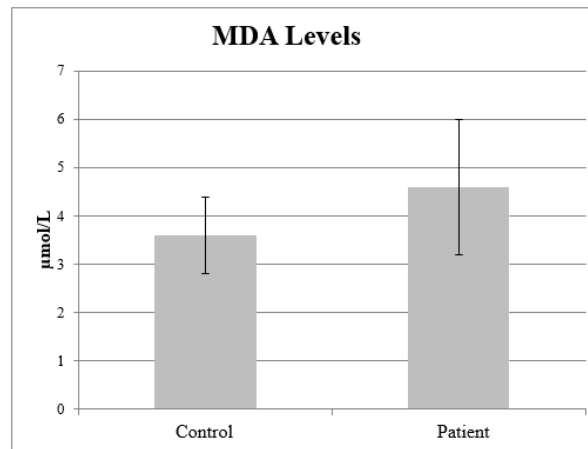


Figure 2. MDA levels change according to the groups (* $p < 0.05$ in comparison to the control group)

4. DISCUSSION

CE is a parasitic disease that has been asymptomatic for many years. Especially in countries where animal husbandry is common, its incidence was found high [1]. This disease affects human and animal health, labour force and productivity negatively and can lead to serious economic losses. For this reason, human and animal studies on this issue are especially important in our country where rural population is widespread and CE shows endemic spread.

Earlier studies showed that NO levels of different tissues were elevated by CE infection via result of host immune responses [23-25]. It has been reported that high amounts of NO produced by activated peritoneal macrophages inhibited the growth and spread of *E. granulosus* in hydatid cyst mice; and also, has been shown in another in-vitro study, NO produced by mouse-derived active macrophages deactivates protoscolerxes of *E. multicoloris* [26, 27]. These results are remarkable in terms of exhibiting the antiparasitic effects of NO over the physiological amounts produced by iNOS as a result of the immune response of the host.

Touil-Boukoffa et al. [17] found that serum NOx levels of patients with hydatid cysts in their liver, brain and spleen were higher than of healthy individuals; and that the increase in oxidized NO derivatives also correlated with the increase in IFN- γ levels that trigger inflammatory responses. In another study, it has been reported serum NOx levels of patients with CE significantly increased compared to healthy controls, and that this increase may be related to the antihydatic effects of NO [13]. In this study, NOx levels in the CE patients group were found to be significantly increased compared to the control group. Increased NO levels appear to be a consequence of the antihydatic activity of NO and the immune response of the host. Our results are consistent with other studies reported in the literature.

It has been claimed that increased free radical production and the resulting oxidative stress play a role in the pathogenesis of CE disease [28, 29]. Various studies have shown *E. granulosus* parasites induce tissue and cell damage by causing an increase in the amount of free radicals in the organs, tissues and cells in which they are located [30, 31]. Bakır et al. [29] have reported that total oxidant status levels and oxidative stress index levels in hydatid cyst patients were increased compared to healthy individuals. It has been reported serum MDA levels of CE patients, were found to be increased significantly compared to control group in two different studies [32, 33]. In another study with patients with lung hydatid cysts, the patient's oxidative stress levels were found to be increased, and this increase was reported to be the result of the patient's immune response [34]. Koltas et al. [35] have investigated that the changes of serum MDA levels in patients with CE. Their results showed that MDA levels were increased in cases of CE. The results of our study also show parallelism with other findings in the literature. When serum MDA levels are evaluated; MDA levels of CE patients were found to be significantly increased compared to control group. Based on these results, it can be said that the respiratory burst, which is a part of the immune response against *E. granulosus*, increases free radical production leading to lipid peroxidation. Additionally, the cumulative effect of reactive oxygen and reactive nitrogen compounds that increased as

a result of the production of NO at high concentrations by iNOS in macrophages stimulated by *E. granulosus* and its turning into NOx derivatives by oxidizing, may have caused lipid peroxidation by affecting cell membranes, which may have triggered the increase of MDA in the serum of CE patients. Consequently, it can be concluded based on the previous studies and the data obtained in this study that the increase of serum NOx and MDA levels in CE patients may be related to the pathogenesis of the disease and the results of the immune response developed against the disease. Considering the results of this study and similar studies in the diagnosis, control and treatment of CE disease, which shows a widespread distribution in our country and affects both human and animal health and developing new perspectives through further studies will be an important achievement in terms of health.

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CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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