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Evaluation of pre- and post-surgical oxidative stress parameters in patients with pulmonary cystic echinococcus

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Abstract. – *Background:* **Hydatid cyst secretes numerous immunomodulatory molecules to the host, and the host reacts these molecules by activating immune response. It is also known that, immune cells generally produce more oxidative products.**

Aim: **In this study, we aimed to evaluate the antioxidant enzyme using catalase (CAT) and oxidant enzyme using lipid hydroperoxide (LOOH) levels in the serum samples before and after the surgical interventions in patients with pulmonary cystic echinococcus (CE).**

Patients and Methods: **Forty patients with pulmonary CE who underwent surgery and 40 healthy individuals were enrolled to the study. Patients were divided in two groups; group 1 (n=40) consisted of patients with pulmonary CE, and group 2 (n=40) consisted of healthy subjects.**

Before and after surgical interventions serum CAT and LOOH levels were measured.

Results: **Compared to group 2, group 1 had significantly lower CAT and higher LOOH levels before surgery (both $p < 0.001$). In group 1; CAT levels were significantly increased and LOOH levels significantly increased after the surgical intervention (both $p < 0.001$).**

Conclusions: **The present study showed that oxidative stress increased in patients with pulmonary CE, may be consequence of immune response of the host, and these levels decreased after the surgical intervention.**

Key Words:

Pulmonary hydatidosis, Immune response, Oxidative stress, Thoracic surgery.

Introduction

Cystic echinococcosis (CE) is a parasitic disease that is endemic in many parts of the world, especially in South America, the Middle East, Africa, Australia, and the Mediterranean region, including Turkey, and is caused by *Echinococcus granulosus*[1,2]. After the penetration of the parasite, the hydatid cyst secretes and exposes numerous immunomodulatory molecules to the host's immune system. In contrast, the host organism react these molecules by activating complement-depending immune response[3,4]. It is also known that, immune cells are particularly sensitive to oxidative stress due to a high percentage of polyunsaturated fatty acids in their plasma membranes, and they generally produce more oxidative products[5].

Although based on these knowledge mentioned above, there have been limited research studies to investigate the oxidative status in patients with CE. These studies have been performed in patients with liver CE[3,6]. No research study has been performed to evaluate the oxidative status in patients with pulmonary CE. By this way, the aim of the present study was to evaluate the antioxidant enzyme using catalase (CAT) and oxidant enzyme using lipid hydroperoxide (LOOH) levels in the serum samples before and after the surgical interventions in patients with pulmonary CE.

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Patients and Methods

Study Design and Patients

This prospective study was conducted at the Thoracic Surgery Department of Harran University School of Medicine, Sanliurfa, Turkey. Prior to subject recruitment, the study protocol was reviewed and approved by the local Ethics Committee, in accordance with the ethical principles for human investigations, as outlined by the Second Declaration of Helsinki, and written informed consent was obtained from all the patients. From September-2009 to July-2011, 40 patients with pulmonary CE who underwent surgery and 40 healthy individuals were recruited to the study.

Patients were divided in two groups: group 1 (n=40) consisted of patients with pulmonary CE, and group 2 (n=40) consisted of healthy subjects. The diagnosis of hydatid cyst was based on chest radiography and computed thorax tomography scanning. All patients were examined with abdominal ultrasonography in order to evaluate the liver. The exclusion criteria were as follows: recent acute infectious illness; any evidence of liver, kidney diseases; diabetes mellitus; malignancy; any other inflammatory, or infiltrative disorder; recent use (within 2 weeks) of any systemic or local drug with antioxidant properties; regular alcohol use or alcohol use within the previous 48 hours. The serum CAT and LOOH levels of all CE patients were analyzed before and the after the surgery. Data were also obtained from the healthy subjects at baseline.

Baseline Definitions and Measurements

Height and weight were measured according to standardized protocols. Body mass index (BMI) was calculated as the weight in kilograms divided by the height in meters squared (kg/m^2). Blood pressure was measured using a mechanical sphygmomanometer. For each subject, after being seated comfortably for 15 minutes, the average of three blood pressure measurements was calculated.

Biochemical Analysis

All of the blood samples were drawn from a large antecubital vein without interruption of the venous flow using a 19-gauge butterfly needle connected to a plastic syringe. Twenty milliliters of blood was drawn, with the first few milliliters discarded. Ten milliliters were used for baseline routine laboratory tests. The residual content of the syringe was transferred immediately to polypropylene tubes, which were then centrifuged at 3000 rpm for 10 minutes at 10 to 18°C. Supernatant serum samples were stored in plastic tubes at –80°C until assayed. For the serum markers of LOOH and CAT were measured.

Measurement of Catalase

Catalase activity was assayed by a method described by Goth[8]; 0.2 ml hemolysate was incubated in 1.0 ml substrate (65 μmol per H_2O_2 in 60 mmol/l sodium-potassium phosphate buffer, pH: 7.4 at 37°C for 60 s. The enzymatic reaction was stopped with 1.0 ml of 32.4 mmol/l ammonium molybdate [(NH_4) 6 $MO_7O_{24} \cdot 4$ H_2O], and the yellow complex of molybdate and H_2O_2 was measured at 405 nm against blank 3. One unit of CAT decomposes 1 μmol of H_2O_2/l min under these conditions. Results were expressed as kU/gHb, which was calculated as follows: CAT (kU/gHb) = [A (sample) – A (blank 1) / A (blank 2) – A (blank 3)] × 271. Blank 1contained 1.0 ml substrate, 1 ml molybdate, and 0.2 ml hemolysate; blank 2 contained 1.0 ml substrate, 1 ml molybdate, and 0.2 ml buffer; blank 3 contained 1.0 ml buffer, 1.0 ml molybdate, and 2.0 ml buffer.

Measurement of Lipid Hydroperoxide

Lipid hydroperoxide amount was measured by a new automated method using xylenol orange. In this method, lipid hydroperoxide oxidizes ferrous ions to ferric ions. The produced ferric ions make a colored mega complex with xylenol orange. The absorbance was measured 570 nm with auto-analyzer (Abbott®, Abbott Park, North Chicago, IL, USA)[7].

Other Variables

Serum urea, creatinine, fasting blood glucose, aspartate aminotransferase, alanine aminotransferase, triglycerides, total cholesterol, and high-density and low-density lipoproteins cholesterol levels were determined using commercially available assay kits (Abbott®, Abbott Park, North Chicago, IL, USA) with an auto-analyzer (Abbott®, Abbott Park, North Chicago, IL, USA).

Statistical Analysis

All statistical analyses were performed using SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL, USA). *Kolmogorov-Smirnov* test was used to test the normality of data distribution. The data were expressed as arithmetic

Table I. Comparison of the demographic and biochemical characteristics of all patients.

	Group 1 (n = 40)	Group 2 (n = 40)	p
Gender, male/female	22/18	19/21	NS[a]
Age, years	31.66 ± 11.11	33.34 ± 9.71	NS[b]
BMI, kg/m^2	24.13 ± 3.43	23.56 ± 2.67	NS[b]
Systolic BP, mmHg	113.32 ± 9.81	116.15 ± 8.94	NS[b]
Diastolic BP, mmHg	70.34 ± 9.06	72.76 ± 8.37	NS[b]
Glucose, mg/dL	84.12 ± 9.45	81.87 ± 10.23	NS[b]
Urea, mg/dL	26.16 ± 5.65	25.38 ± 4.31	NS[b]
Creatinine, mg/dL	0.71 ± 0.19	0.69 ± 0.26	NS[b]
ALT, U/mL	27.12 ± 5.32	26.08 ± 4.54	NS[b]
AST, U/mL	25.54 ± 4.87	26.76 ± 4.41	NS[b]
Total cholesterol, mg/dL	212.45 ± 19.84	201.31 ± 24.64	NS[c]
LDL cholesterol, mg/dL	134.73 ± 32.13	139.62 ± 28.72	NS[c]
Triglyceride level, mg/dL	195.91 ± 44.36	201.47 ± 52.41	NS[c]
CAT, U/L	6.74 ± 1.74	10.08 ± 1.86	0.001[b]
LOOH, μmol H_2O_2 Eqv./L	36.58 ± 10.26	8.28 ± 0.58	0.001[b]

All measurable values were given with mean ± standard deviation. NS: non-significant, ALT: alanine aminotranferase, AST: aspartate aminotransferase, LDL: low density lipoproteins, CAT: catalase, LOOH: lipid hydroperoxide. By Chi-square[a], Independent sample *t* test[b] and Mann Whitney-U[c] tests.

means and standard deviations. The *chi-square test* was used to compare the categorical variables between groups. *Independent sample T-test* and *Mann Whitney-U* tests were used for comparison of continuous variables between two groups. *Paired t-test* was used to analyze changes within the CE patients. Two-sided p value < 0.05 was considered statistically significant.

Results

The clinical, biochemical and demographic characteristics of the study groups are presented on Table I. There were no significant differences in gender, age and biochemical values between the control and CE patients ($p > 0.05$ for all). Compared to group 2, group 1 had significantly lower CAT and higher LOOH levels at baseline (both $p < 0.001$). In group 1; CAT levels were significantly increased and LOOH levels significantly increased after the surgical intervention (both $p < 0.001$).

Discussion

To the best of our knowledge, this is the first report to evaluate the oxidative status in patients with pulmonary CE. The main findings of the present study showed that (1) the oxidative stress increased in patients with pulmonary CE, and (2) these levels decreased after the surgical intervention.

The host-parasite relationship is interactive and the outcome of infection depends on the balance achieved by the combination of the different variables involved with the host immuni-

Table II. Comparison of CAT and LOOH levels in Group 1 after the surgical intervention.

	Before surgery (n = 40)	After surgery (n = 40)	p[a]
CAT, U/L	6.74 ± 1.74	8.56 ± 2.10	0.001
LOOH, μmol H_2O_2 Eqv./L	36.58 ± 10.26	19.73 ± 6.48	0.001

All measurable values were given with mean ± standard deviation. CAT: catalase, LOOH: lipid hydroperoxide. By Paired sample *t* test[a].

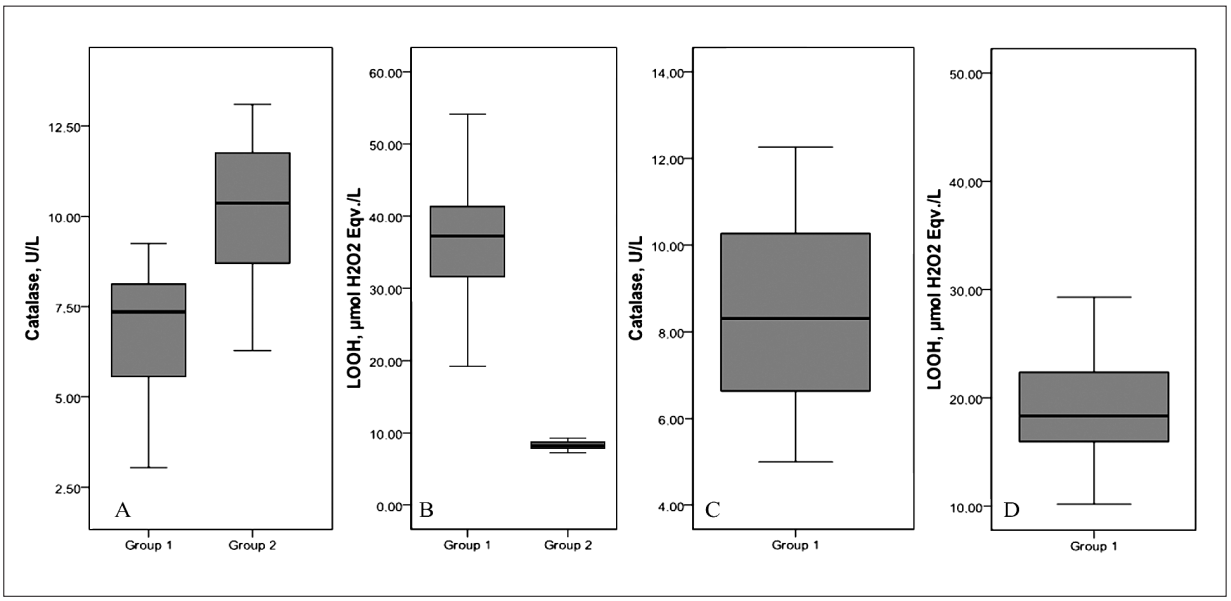

Figure 1. *A*, Graph demonstrating the CAT levels at baseline among two groups. *B*, Graph demonstrating the LOOH levels at baseline among two groups. *C*, Graph demonstrating CAT levels after the surgical intervention. *D*, Graph demonstrating LOOH levels after the surgical intervention.

ty and the parasite avoidance strategies[9]. The main feature of this relationship is the coexistence of the chronic infection with detectable humoral and cellular responses against the parasite[10-12]. Helminths penetrate and establish themselves in the host tissues, incorporate metabolites from the host, and modulate the host immune response by developing a variety of effective strategies[13,14]. Some of these strategies are passive, whereas others involve active manipulations of the host's defensive responses. Namely, parasite-derived substances play an important role in initiating or maintaining the parasite's advantage, directly suppressing the function of certain subsets of immune cells as well as stimulating other cell populations related to immunopathology[15-19].

Little is known about parasitic molecules that behave as immunomodulatory antigens and the mechanisms that they umune response[20]. Some studies demonstrated that hydatid cyst produces and secretes significant quantities of molecules that modulate the immune responses; these include both humoral and cellular immune response against the parasite[21,22]. It is also known that antioxidant system, which is responsible for minimizing oxidative cell damage in the body caused by free radicals, can be produced by exogenous sources or not only be a product of physiological metabolic processes, such as can be produced by immune response. Thus, chronic inflammation is closely associated with oxidative stress[23-25].

In literature, a limited number of research studies have been performed to elucidate this relationship. Lilic et al[3] showed that the activity of the glutathione peroxidase levels were significantly lower before the surgery and glutathione-S-transferase levels were significantly higher after the surgery in the plasma in patients with liver EC. Koltas et al[6] investigated malondialdehyde levels in patients with liver CE and have found higher levels in respect to healthy controls. In our study, compared to healthy subjects, we found decreased CAT levels and increased LOOH levels in patients with pulmonary CE. In addition, these levels were reversed after the surgery.

In conclusion, findings of the present report demonstrate that oxidative stress increased in patients with pulmonary CE. Although not definitively, it may be a consequence of immune response of the host, and the treatment of CE decreases these levels. Relatively small sample size is a major limitation of the present work and assessing several other markers of oxidative stress might add to the value of our manuscript; however we could only have opportunity to analyze CAT and LOOH levels for this study. Therefore, future large-scale prospective cohort investigations are needed to clarify this issue.

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