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Antioxidant Enzyme Status in Human Cystic Echinococcosis

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The objective of this study to investigate the role of antioxidant enzymes against toxic reactive oxygen species in human Hydatid disease (HD). This study took place in the Faculty of Medicine, University of Cukurova, Balcali Hospital in Adana, Turkey, from March 2004 to October 2005. The study was conducted on patients with cyctic echinococcosis (CE) before the surgical treatment and compared with healthy controls. Cyctic echinococcosis was determined by ELISA and western blotting method in serum samples. We assayed catalase (CAT) and superoxide dismutase activities (SOD) measured of 28 subjects and matched in 45 healthy controls. CAT and SOD activities of the patients and control group were found as $15.18 \times 10^4 \pm 3.25 \times 10^4$ IU/mg Hb, 2.40 ± 0.27 U/mL and $17.84 \times 10^4 \pm 2.61 \times 10^4$ IU/mg Hb, 3.63 ± 0.41 U/mL, respectively. Present results showed that there was a significant decrease in CAT ($p < 0.05$) and SOD ($p < 0.001$) activities of patients group as compared with that of the controls. There is an increase in oxidative stress in cystic echinococcosis. Despite this stress, the antioxidant system is deficient and adequate, in patients with cystic echinococcosis could be a defense system promotes the regulation and expression of these enzymes.

Key Words: Cystic echinococcosis, Oxidative stress, Catalase, Superoxide dismutase.

INTRODUCTION

Echinococcosis is a cosmopolitan zoonosis caused by adult or larval stages of cestodes belonging to the genus Echinococcus (family Taeniidae). Larval infection (cystic echinococcosis, hydatid disease, hydatidosis) is characterized by long-term growth of metacestode (hydatid) cysts in the intermediate host. The two major species of medical and public health importance are *Echinococcus granulosus* and *E. multilocularis*, which cause cystic echinococcosis (CE) and alveolar echinococcosis (AE), respectively[1]. Cystic echinococcosis exists throughout the world and in many regions it is a major public health and economic problem[2]. Aerobic organisms possess antioxidant defense systems that deal with reactive oxygen species (ROS)

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produced as a consequence of aerobic respiration and substrat oxidation. Small amount of ROS, including hydroxyl radicals, superoxide anions and hydrogen peroxide, are constantly generated in aerobic organisms in response to both external and internal stimuli[3-5]. High doses and/or inadequate removal of ROS result in oxidative stress, which may cause damage to all major classes of biological macromolecules leading to protein oxidation, lipid peroxidation, depolymerization of polysaccharides, DNA modifications/strand breaks, etc. To counter these destructive processes, all aerobic organisms have developed extensive, multi-layered enzymatic and non-enzymatic systems, which act to prevent and repair the ROS-derived damage[6]. It has been postulated that antioxidant enzymes are essential for parasites to defend themselves against ROS generated by macrophages, neutrophils and eosinophils of the host, in addition to their normal functions in aerobic organisms. These enzymes may be particularly important for long-lived parasites that are involved in chronic infections, such as parasitic nematodes and trematodes[7]. In recent years research has focused in assessing the possible role of the highly reactive oxygen free radicals in the pathogenesis of parasitic infections[8]. The enzymatic and non-enzymatic antioxidant defenses include superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), ascorbic acid, α-tocopherol, glutathione (GSH), β-carotene and vitamin A. Although the antioxidant enzymes have been extensively characterized in parasitic nematodes and trematodes, few studies have been carried out on cestodes[9]. In this study, it is aimed to investigate the antioxidant status in patients with cystic echinococcosis before the surgical treatment and compared with healthy controls.

EXPERIMENTAL

This study was conducted on patients with cystic echinococcosis (CE) before the surgical treatment from March 2004 to October 2005 at the Faculty of Medicine, University of Cukurova, Balcali Hospital in Adana, Turkey. Anti-*E. granulosus* antibodies were determined by Echinococcosis ELISA and Echinococcosis Western blotting (WB), Euroimmun, Germany, according to the protocols established by the manufacturer. We assayed CAT and SOD activities measured of 28 subjects (15 men and 13 women) aged between 27-66 years and matched in 45 healthy controls. All patients were liver CE at least 2 years. None of them were smokers, had any known pathologies and taking steroids or medications and had any infectious disease such as hepatitis, HIV or parasitic diseases other than CE. Serum samples for controls were obtained from healthy subject. These individuals were free from infections. Their investigations were carried out by regular laboratory check-up analysis. All subjects fasted after midnight before blood collection the next morning. Twenty eight patients and 45 controls were examined in this study. The mean age of the patients, which consisted of 15 men and 13 women were 42.7 ± 11.5 years and 40.7 ± 11.3 years. The mean age of the controls, which included 24 men and 21 women were 42.1 ± 13.1 years and 40.8 ± 11.7 years. All venous blood samples taken between 8 and 9 am after 8 h of fasting were collected in polystyrene tubes and vacutainers containing heparin.

The study protocol was reviewed and approved by the Faculty of Medicine Ethics Committee of the University of Cukurova, Adana, Turkey and informed consent was obtained for each participant.

Biochemical assay

Preparation of erythrocytes: Heparinized blood was centrifugated at 3000 rpm for 15 min and plasma and leukocytes were discarded. Erythrocytes were washed at least three times with 0.9 % sodium chloride solution. The cells were suspended to 40 % by volume in the same solution.

CAT activity assay: CAT activity was determined according to the Lartillot *et al.*[10] which is a modification of the method described by Bergmeyer[11]. CAT activity was measured spectrophotometrically at 240 nm using a spesific absorption coefficient of 0.0392 cm^2 μmol $H_2O_2^{-1}$.

SOD activity assay: SOD activity was determined according to the method of Sun *et al.*[12]. This assay for SOD activity involves inhibition of nitroblue tetrazolium reduction, with xanthine-xanthine oxidase used as a superoxide generator. The production of formazan was determined spectrophotometrically at 560 nm.

Statistical analysis: Statistical analysis was performed using SPSS software package (Version 11.0 for Windows). The data were expressed as mean ± standard deviation (SD). For comparison of 2 groups of continuous variables, independent sample t-test was used. A probability value of less than 0.05 indicated a statistically significant difference.

RESULTS AND DISCUSSION

Table-1 shows CAT and SOD activities of patients infected with cystic echinococcosis and controls. The difference between CAT activities of patients and controls was statistically significant both for women and men ($p < 0.05$). The difference between SOD activities of patients and controls was also statistically significant both for women and men ($p < 0.001$).

TABLE-1
CATALASE (CAT) AND SUPEROXIDE DISMUTASE (SOD) ACTIVITIES OF PATIENTS INFECTED WITH CYSTIC ECHINOCOCCOSIS (CE) AND CONTROL GROUP

Parameters	Age (years) Mean ± SD	CAT activity (IU/mg Hb) Mean ± SD	SOD activity (U/mL) Mean ± SD
Patients			
Female (n = 13)	40.7 ± 11.3	$15.13 \times 10^4 \pm 3.19 \times 10^4$	2.38 ± 0.26
Male (n = 15)	42.7 ± 11.5	$15.25 \times 10^4 \pm 3.30 \times 10^4$	2.43 ± 0.29
Total (n = 28)	41.6 ± 11.2	$15.18 \times 10^4 \pm 3.25 \times 10^4$	2.40 ± 0.27
Controls			
Female (n = 21)	40.8 ± 11.7	$17.73 \times 10^4 \pm 2.53 \times 10^4$	3.59 ± 0.43
Male (n = 24)	42.1 ± 13.1	$17.96 \times 10^4 \pm 2.69 \times 10^4$	3.67 ± 0.40
Total (n = 45)	41.2 ± 11.7	$17.84 \times 10^4 \pm 2.61 \times 10^4$	3.63 ± 0.41

Reactive oxygen species (ROS) are an inescapable consequence of aerobic metabolism. The sequential reduction of oxygen to water produces superoxide radical and hydrogen peroxide. These species may have potentially deleterious effects, since they can interact with each other and with cellular components, leading to radical chain reactions and ultimately to cell death[13,14].

High concentrations of ROS can have serious effects on membrane lipids, nucleic acids and proteins. Therefore, organisms have evolved both antioxidant systems for protection against these ROS and enzymes to repair oxidatively damaged molecules[15,16]. Parasitic organisms have an additional requirement for these enzymes, since they may also be exposed to oxidizing agents derived from host effector cells[17]. Catalase and SOD are primary intracellular, antioxidant defense mechanisms to cope with increased oxidant stress[13,17-19].

The decreased catalase activities have been shown in *P. berghei* and *P. falciparum* infections. Erel *et al.*[20] found decreased erythrocyte catalase activity in patients with vivax malaria. On the other hand, Buldanlioglu *et al.*[21] didn't find significant differences in erythrocyte SOD activities in patients with Behcet's diseases and control groups. In our previous study, we found that serum MDA levels were significantly increased in patients with cystic echinococcosis when we compared their values with controls[22]. This was the first study to characterize the relationship between cystic echinococcosis and catalase and SOD activities. In this study, we found that the decreased erythrocyte CAT and SOD activities in patients with cystic echinococcosis when we compared their activities with controls (Table-1). During literature survey, no report is available for study related to CAT and SOD activities of cystic echinococcosis. Therefore, it is not possible to compare present results with the previous studies. The results of the study suggest that one of the main reasons for decreased CAT and SOD activities in patients with cystic echinococcosis could be increased lipid peroxidation. However, neither could any significant correlation be found between CAT and SOD activities of both females and males for patients with cystic echinococcosis and control groups.

In conclusion, the results of our study suggested that one of the main reasons for this low CAT and SOD activities in patients infected with cystic echinococcosis could be a defense system promotes the regulation and expression of these enzymes. Present findings have shown that antioxidant enzyme activities are effected by cystic echinococcosis.

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