Evaluation of Oxidative Stress and Antioxidants in Iraqi Patients with Hydatid Disease

Ahmed A. Mohammed

Branch of Clinical Laboratory Sciences, College of Pharmacy, Mustansiriyah University, Baghdad-Iraq

Abstract

Antioxidants and oxidative stress status are clinically important in the detection of many diseases. In order to estimate the role of oxidative stress in hydatid disease pathogenesis, the antioxidant levels and oxidative stress status were examined in the patients of hydatid cysts. Thirty patients having active hydatid cyst of *Echinococcus granulosus*, previously diagnosed by X-ray, were adopted as patients' group. Additionally, 25 healthy individuals who did not have hydatid infection or any other parasitic infection constituted the control group. Antioxidant status and oxidative stress levels were determined using biochemical tests including plasma Malondialdehyde (MDA) level, and erythrocyte Catalase (CAT), Glutathione Peroxidase (GSH-Px), Superoxide Dismutase (SOD), Glutathione-S-Transferase (GST) activity, in addition to erythrocyte Glutathione concentration GSH-conc. The statistical evaluation of plasma MDA levels showed significantly higher levels in hydatid patients than in healthy controls, while erythrocyte SOD, GSH-Px, CAT, GST and GSH levels were significantly declined in hydatid patients compared to the control which can be used as diagnostic markers in the laboratory diagnosis of the disease. To conclude, hydatid patients show elevated oxidative stress status, and therefore, the antioxidant therapy should be considered in addition to the routine medicines in this group of patients.

Keywords: *Hydatid disease, Echinococcus granulosus, Antioxidant, Oxidative stress.*

Introduction

Hydatid disease, or cystic echinococcosis (CE), is an endemic cosmopolitan zoonosis. It is developed from an infection with the larval stage of the tapeworm *Echinococcus granulosus*. The disease is transmitted to human by oral intake of parasite eggs expelled in the dogs' feces, the main definitive host, which can result in single or multiple hydatid cysts [1-3]. The liver and lungs are noticeably most affected organs; however, the brain, kidneys, spleen, heart as well as bones can also be infected via lymphatic and hematogenous routes [4,5]. Indeed, earlier diagnosis leads to a highly successful rate of treatment, yet, it is difficult to be diagnosed clinically due to the variable signs and symptoms, which correlate with the infected organs. However, successful diagnosis requires a combination of different techniques including physical examination, imaging and serological investigations [2,6-8].

Free reactive oxygen radicals such as nitric oxide (NO), hydrogen peroxide H_2O_2 and hydroxyl as well as superoxide radicals are strongly reactive molecules which are produced during the normal metabolism or after exposition to ecological pro-oxidants. Overproduction of free radicals causes a serious chain reaction which can destroy the lipids, nucleic acid, proteins and other cellular compounds [9]. The body fights the excessive free radicals via its antioxidant defence system, which comprised of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and by nutritional antioxidants for instant vitamins E and C and also ceruloplasmin [10]. However, oxidants and antioxidants level in healthy individuals are at a good balance.

The oxidant and antioxidants balance will be disrupted in the prolonged exposure to *E. granulosus* antigens due to the continuous immune reactions of the parasite products with oxygen radicals during the parasitic infection, which increases the oxidant stress and ultimately leads to oxidative damage, which in turn has an impact in the complications of the disease as in various other diseases [11].

Cells containing antioxidant functions have a great

influence in the protection against reactive radicals by fighting the oxidative damage of the basic structural elements of cells such as lipids, proteins and nucleic acids [12], and ultimately, it leads to cell death via necrosis or apoptosis [13]. However, it is noteworthy that the antioxidant system is composed of antioxidant enzymes including superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glucose 6-phosphate dehydrogenase (G6PD); metal binding proteins which are non-enzymatic substances such as transferrin, ceruloplasmin and albumin; vitamins such as alpha-tocopherol and beta-carotene, as well as trace elements including iron, copper and zinc [12].

The hydatid cyst causes phagocytic cell activation during the host immunosuppressive response, resulting in the release of reactive oxygen species (ROS) and reactive nitrogen products from the macrophages in response to the cyst pathogenicity [13]. Although studies of oxidative stress have been reported in humans [14], camels, sheep and cattle [15], there is no study performed in the patients of cystic echinococcosis that studied together the plasma malondialdehyde (MDA) level and erythrocyte CAT, SOD, GST, GSH-Px activities and erythrocyte GSH-conc. Therefore, the goal of this study is to estimate whether oxidative stress has any role in the pathogenicity of hydatid infection by examining the levels of oxidative stress and antioxidant status in the patients of hydatid disease.

Method

Subjects

Plasma and erythrocyte samples were obtained from the Hospital of Specialized Surgeries in Baghdad-Iraq from the patients of hydatid cysts. Thirty patients and 25 controls were considered in the current study. The controls were free of any medication for at least one week before and during the study.

Biochemical tests

A- Plasma MDA assay: the plasma MDA was assayed according to Ohkawa *et al.* (1979) with the modification done by Hirayama *et al.* (2000). Every two acid-reactive molecules of Thiobarbituric acid (TBA) will react with one molecule of plasma MDA to produce a reddish chromogen which can be detected at 532 nm wave length.

B- SOD assay: SOD was investigated according to Kakkar *et al.* (1984). In this method, the resulted oxygen from the photo-reduction of riboflavin will inhibit the reduction of nitro blue tetrazolium (NBT). Fifty percent of inhibition known as one unites of SOD activity.

C- CAT assay: The activity of CAT was estimated according to Eaton *et al*. (1972) method. CAT catalyzes the H_2O_2 to H_2O_2 and O_2. The rate of H_2O_2 decomposition by the action of CAT is measured photometrically at 230nm. To increase the stability of the hemolysate, the ethanol should be added to prevent the breakdown of the catalase and H_2O_2. After applying 50µl of tris buffer, 900µl H_2O_2 and 30µl of H_2O, the system incubated at 37˚C for 10 minutes, and the hemolysate was applied following 10 minutes. The decrease of OD is measured versus a blank at 412 nm.

D- GSH-Px assay: GSH-Px has assayed according to Paglia and Valantine (1967) with some modifications from Hopkins and Tudhope *et al.* (1973) and Pleban *et al.* (1982). The recycling procedure of the estimation of the activity of GSH-Px relies on the oxidation of the glutathione to glutathione reductase by the GSH-Px in the presence of NADPH and exogenous GSSG which regenerates GSH for GSSG [16]. The enzyme level was monitored by following the decline in the absorbance at 340nm as an indicator of NADPH exhaustion [10].

E- GST assay: GST was assayed by the procedure of Habig *et al.* (1974) with some modifications from Carmagnol *et al.* (1981). The enzyme concentration was investigated through monitoring the absorbance difference at 340nm. A complete assay mixture without glutathione was used as a reference.

F- GSH: Measuring the level of GSH in the erythrocyte was performed as prescribed by Virgil *et al*. (2000) and Beutler *et al.* (1963). Virtually, all the non-protein sulfhydryl groups of erythrocytes are found as a reduced GSH. In addition, 5, 5-Dithio bis 2-nitrobenzoic acid (DTNB) is a disulphide chromogen that is readily reduced by the sulfhydryl compounds to an intensely yellow compound. The reduced chromogen absorbance is measured at 412nm.

Statistical Analysis

The results were analyzed by t-test. P value ≤ 0.05 was considered significant. Data analysis was performed using statistical software (IBM SPSS Statistics 20).

Results

The mean ± SD of plasma MDA level (µmol/L Hb) was significantly increased in the patients with hydatid disease compared with that in healthy subjects ($p < 0.005$). Moreover, the mean ± SD of erythrocyte SOD activity was decreased in hydatid cyst patients compared with healthy control ($p \leq 0.001$).

Additionally, the mean ± SD of erythrocyte CAT activity (U/mg Hb) in hydatid patients was lower than those in the healthy control ($p < 0.001$).

Moreover, the mean ± SD of erythrocyte GSH-Px activity was significantly declined in group 2 of hydatid patients compared with healthy control ($p < 0.005$). However, hydatid patients had a significantly lower mean ± SD of erythrocyte GST activity (U/g Hb) compared with healthy individuals ($p \leq 0.005$).

Similarly, the mean ± SD of erythrocyte GSH-conc. (µmol/g Hb) of hydatid patients was also lower than that of the control ($p \leq 0.001$).

Table (1): The statistical analysis of the investigated tests in the groups of the patients and the control.

Parameter	Normal control (n = 25)	Hydatid patients (n = 30)	p-value*
Plasma MDA level (µmol/L Hb)	4.32 ± 1.45	8.55 ± 2.5	< 0.005
Erythrocyte SOD activity U/mg Hb	4.04 ± 0.63	1.7 ± 0.46	≤ 0.001
Erythrocyte CAT activity U/mg Hb	66.9 ± 5.2	43.9 ± 5.23	< 0.001
Erythrocyte glutathione peroxidase activity GSH-Px U/g Hb	33.01 ± 1.09	20 ± 0.88	< 0.005
Erythrocyte GST activity U/g Hb	2.2 ± 0.7	0.87 ± 0.29	< 0.005
Erythrocyte GSH-conc. µmol/g Hb	7.2 ± 0.98	3.54 ± 0.8	< 0.001

**P*-value from student's t-test.

Discussion

The current study has investigated the levels of plasma MDA, erythrocyte CAT, SOD, GST and GSH-Px activities as well as the erythrocyte GSH in cystic echinococcosis patients to find if they can be used as indicators to the oxidative stress and antioxidant status in the patients of hydatid disease. The statistical evaluation has reached significant differences in the level of plasma MDA, CAT, SOD, GST and GSH-Px activities as well as GSH concentration in the patients with hydatid cysts compared to healthy individuals.

To begin, it has been found that the SOD concentrations were enormously decreased in the patients having hydatid cysts compared to healthy individuals. In addition, it has demonstrated that MDA, a non-enzymatic antioxidant and a biomarker of lipid peroxidation, was extremely higher in hydatid cysts patients compared to normal control. Importantly, lipid peroxidation is a main deteriorating change in unsaturated fatty acids of the cell membranes induced by the excess free radicals [9]. Similarly, it was shown that MDA level has been adopted as a biomarker to the extent of free radicals' production, oxidative stress and tissue damage and it was increased significantly in the hosts infected with hydatid cysts [14,27]. Undoubtedly, the over-generation of MDA is clearly confirming the accumulation of reactive oxygen radicals and the incidence of oxidative stress, especially in hepatic hydatid infection. Therefore, the estimation of MDA levels is a reliable approach to evaluate the percentage of peroxidative damage of cell membranes because it serves as the most abundant aldehyde

produced as a secondary product [28]. Moreover, lipid peroxidation resulted from excess free radicals leads to the disarrangement and, subsequently, disruption of cell membranes which leads to necrotic death [15]. Deger *et al.* (2008) reported a positive correlation between the serum MDA and aspartate transaminase, an important predictor of hepatic damage, in the sheep with cystic echinococcosis. Thus, it has suggested that the oxidative damage could have an impact on the liver damage in the sheep with hydatid cysts.

Additionally, the current study revealed a significantly lower reserve of CAT, GSH-Px, GST activities and erythrocyte GSH in the hydatid patients, which is consistent with the previous reports indicated that the concentrations of antioxidant enzymes including the GSH, glutathione peroxidase and SOD were prominently declined in hydatid patients [14]. Surely, glutathione transferases and enzymes in the cellular metabolic detoxification process are involved in the removal of genotoxic and cytotoxic compounds and in the protection against the oxidative damage as well [30]. The cellular metabolic detoxification process depends primarily on the reduction and hydrolysis reactions due to the low cytochrome P450 efficiency. Consequently, the cytosolic GSTs became the major types of detoxifying enzymes in helminths [31].

The examination of oxidative stress level and antioxidant status in the patients of hydatid disease and their role in the pathogenesis of the hydatid cyst has not investigated before by determining the oxidative stress level and antioxidant status using biochemical tests included the levels of plasma MDA, CAT, SOD, GST, GSH-Px activities and the erythrocyte GSH-conc in hydatid cyst patients.

Conclusions

The current study showed that the oxidative stress increases in the patients infected with hydatid cysts, which suggest that it might lead to the increase in the tissue necrosis and inflammation, while the decrease in the antioxidants level can be associated with low stimulation of cell-mediated immune response. Therefore, the antioxidant therapy should be considered in addition to the routine medicines in this group of patients.

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