



# Comparison of Serum RSH, Immunoglobulin Levels and MPO Activity in Patients with Cystic Echinococcosis in Turkey

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## ABSTRACT

The aim of this study was to elucidate the effect of larval form of *Echinococcus granulosus* on human serum antioxidant status and MPO activity and also serum immunoglobulin levels. For this research, sera of 361 patients were received who applied to Refik Saydam Hıfzısıhha and Etlik SSK Hospital between 2003 and 2005 are suspected of the having Cystic Echinococcosis. It was found that 201 (55.7%) out of 361 patients were seropositive for the disease. Special reagent in the positive and control measured IgG, IgA and IgM levels. Total sulfhydryl groups (RSH) levels and the myeloperoxidase (MPO) activity was investigated by spectrophotometric methods. Although IgA levels increased in seropositive patients, IgG and IgM levels were not change in seropositive patient's serum. Although MPO activity increased, RSH levels weren't change seropositive patient's serum. CE may alter immunoglobulin levels and antioxidant status of both female and male patients with *E. granulosus* infection.

**Key Words:** *Cystic Echinococcosis (CE), Indirect Hemagglutination (IHA), Immunoglobulin (Ig), RSH, Myeloperoxidase (MPO).*

## 1. INTRODUCTION

Cystic Echinococcosis (CE) is a zoonotic parasitic disease of human by the larval stage, genus *Echinococcus*. The parasite is found worldwide,

especially in undeveloped and developing countries [1]. CE is an endemic problem that is seen in both human and animals in all over Turkey and the spread of infection is more wide spread in East and Southeast Anatolia regions than the other parts of Turkey [2]. In this study, apart

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from the other part of Turkey, we examined the changing status of CE in central Anatolia. As the development of CE results in decrease in production (milk, meat, wool, etc.) and discard of severely infected organs, it can have significant negative impact on the economy [1, 3-5]. Additionally, the infection causes high treatment and hospitalization costs and loss of labour in human [6-8].

The diagnosis of CE mainly depends on radiological and immunological techniques. Serological tests for diagnosing hydatid disease in people living in areas where the disease is endemic are useful because of its low cost and ease of performance [9]. Indirect hemagglutination test (IHA) may detect the presence of antibodies to the helminthes parasite. The IHA test is one of the most sensitive serological tests for the diagnosis of CE. It is performed routinely as a screen and its sensitivity was reported as 66-100 % [10]. The Echinococcus Fumouze (Laboratoires Fumouze, France) is an easy-to-use test, which provides results in 2 hours. The test was extensively evaluated with sera from patients with proven CE and control sera.

CE stimulates a cascade of cellular and humoral immune responses in human. As a proof of humoral immune determines the serum antibody levels [11]. Hernandez et al. (2005) reported that serum samples (305) were analyzed by ELISA to determine specific IgG against crude antigens from *Echinococcus granulosus* [12]. However, in fistula growth lung hydatid cyst major specific antibody was IgA [13].

In organism, increased chronic infections like CE may alter the balance between oxidant and antioxidant system. When this balance was broken, oxidative stress developed. Free radicals such as hydroxyl ( $\cdot\text{OH}$ ), superoxide radical anion ( $\text{O}_2^{\cdot-}$ ), alkoxy ( $\text{RO}\cdot$ ) and peroxy ( $\text{ROO}\cdot$ ) radicals cause oxidative stress. These radicals are the important implement of normal and abnormal biological processes [14].

These free radicals convey negative potential effect on macromolecules such as DNA, lipids and proteins [15]. In the body, enzymatic and non-enzymatic antioxidant defense systems are present in cells to protect the membranes and other cell organelles from the damaging effects of free radicals [16]. Various endogenous agents, such as sulphhydryl (RSH) non protein compounds, limit the production of oxygen-derived free radicals and could be related with cellular protection [17]. Sulphydryl groups are important elements of the antioxidant defense in the organism. It has been known that administration of alpha-tocopherol (only a single injection) to patients with hydatid disease of the liver in preoperative period beneficial effect on the decreasing lipid peroxidation [18].

It has been known that MPO activity increased only in the early stage of the infection by helminthes [19]. MPO release into the phagosome via degranulation process, react with hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), formed by the respiratory burst and a halide, particularly chloride. The initial product of the MPO-  $\text{H}_2\text{O}_2$ -chloride system is hypochlorous acid (HOCl) and subsequent formation of chlorine, chloramines and hydroxyl radicals and singled oxygen proposed. These same toxic agents can be released to the outside of the cell, where they may attack normal tissue and thus contribute to the pathogenesis of disease [20].

In the lights above, we aimed to investigate the effect of this disease on RSH, immunoglobulin levels and MPO activity in seropositive patients with *Echinococcus granulosus*.

## 2. EXPERIMENTAL

### 2.1. Materials

TBA, TCA, HCl, BHT, Tris, EDTA, SDS, DTNB,  $\text{H}_2\text{O}_2$ , Ortodianizidine, Sodium Phosphate Buffer, HCl, BSA, Folin, NaOH,  $\text{Na}_2\text{CO}_3$ ,  $\text{CuSO}_4$  and Na tartrate used for the study were purchased from Sigma Chemical Co (St. Louis MO, USA).

In this study, sera of 361 patients were received who applied to Refik Saydam Hıfzısıhha and Etlik SSK Hospital between 2003 and 2005 are suspected of the having CE. Echinococcosis Fumouze reagent allows the detection of anti-*Echinococcus granulosus* antibodies present in the serum, though an indirect hemagglutination (IHA) reaction. In spite of much progress in the use of various imaging techniques for the diagnosis of human hydatidosis, immunodiagnostic confirmation of the infection is frequently required before surgical intervention or institution of chemotherapy [11].

### 2.2. Methods

**IHA test:** The Echinococcosis Fumouze kit (Laboratoires Fumouze) was used according to the instructions of the manufacturer, with a modification that the U-shaped plates provided by the manufacturer were replaced with V-shaped microtiter plates (Greiner Laboratories, Alphen, The Netherlands), as the latter allowed better readability of the results: absence of agglutination was observed with the V-shaped wells as a clear, sharp, dark spot instead of the more or less wide ring seen with the original U-shaped well [21]. Double-aldehyd-stabilized red blood cells were sensitized with the optimum sensitizing dose of hydatid cyst fluid antigen. The hydatid antigen-sensitized, double-aldehyd-stabilized cells were treated with serum to detect antibodies. The hemagglutination pattern of agglutinated red blood cells was noted after overnight incubation at  $4\text{ }^\circ\text{C}$ . An antibody titer of 1:128 in serum was considered to be diagnostic of the disease.

**Immunoglobulin Levels:** Immunoglobulin levels were measured by means of nephelometer, using an immunochemical reagent (Bn System, Dade Bahring, USA). The proteins contained in the human serum from immune complexes with specific antibodies. These complexes scatter a beam of light passed through the sample. The intensity of the scattered light is proportional to the concentration of the relevant protein in the sample. The result is evaluated by comparison with a standard of known concentration.

**Plasma RSH Levels:** As an important antioxidant the RSH levels were determined by the modified Ellmann method. RSH levels were given as nmol/ml [22].

**MPO Enzyme Activity:** MPO activity was determined by Glowick and Kaplan method in patients who are suspected of the having CE [23].

**Protein Detection with Lowry Method:** Protein detection was measured by the Lowry method. Plasma MPO activities were given with U/ml protein [24].

**Statistics:** All values were showed with arithmetic average and standard deviation. The results were evaluated by ANOVA and Mann Whitney U tests. A p value <0.05 was considered significant.

### 3. RESULTS

#### 3.1. IHA Tests Results

In IHA test studied on 361 patients, and 201 (55.7%) of cases had positive IHA test for *E.granulosus*. When results were examined with regard to sex, seropositive

female was 66.7 % and also seropositive male was 33.3 % in the seropositive group (Table 1). The highest titre 1/1280 (46.3%) was found in all patients' sera (Table 2). The obtained values from data were compared with CE presence of the patients and found to be 100 % parallel to 1/1280 titers.

100 patients who underwent CE surgery at different times before this study were involved organ was the liver (30 patients; 68.18%), followed by the lung (6 patients; 13.63%) and in other organs in the remaining patients.

**Table 1.** Percentage of IHA test results in patients' sera

| PATIENTS | Seropositive   | Controls       |
|----------|----------------|----------------|
| Male     | 33.3 (67/201)  | 36.9 (59/160)  |
| Female   | 66.7 (134/201) | 63.1 (101/160) |
| Total    | 55.7 (201/361) | 44.3 (160/361) |

**Table 2.** Percentage of seropositive patients with titers of IHA

| Titer of IHA | Serorositive Male<br>(n=67) | Seropositive Female<br>(n=134) | Total Seropositive<br>(n=201) |
|--------------|-----------------------------|--------------------------------|-------------------------------|
| 1:160        | 20.9 (14/67)                | 14.2 (19/134)                  | 16.4 (33/201)                 |
| 1:320        | 10.5 (7/67)                 | 13.4 (18/134)                  | 12.4 (25/201)                 |
| 1:640        | 11.9 (8/67)                 | 11.2 (15/134)                  | 11.5 (23/201)                 |
| 1:1024       | 14.9 (10/67)                | 12.7 (17/134)                  | 13.4 (27/201)                 |
| 1:1280       | 41.8 (28/67)                | 48.5 (65/134)                  | 46.3 (93/201)                 |

#### 3.2. Immunoglobulin Levels

The potential roles of specific antibodies of different immunoglobulins (IgG, IgA, IgM) in serological diagnosis of CE were investigated by a specific reagent with nephelometer assay based on N Antiserum.

Presences of all Ig were demonstrated in all sera from 361 patients with CE. There was no difference in IgG and IgM between controls and seropositive groups. IgA levels were higher seropositive male patients than control male. In addition, total seropositive group was found increased IgA levels when compared to the total control (Table 3)

**Table 3.** IgG, IgA and IgM levels of patients Cystic Echinococcosis

| PATIENTS             | IgG (g/L) | IgA (g/L) | IgM (g/L) |
|----------------------|-----------|-----------|-----------|
| Control Male (59)    | 11.1±3.6  | 1.9±1.0   | 1.0±0.8   |
| IHA (+)Male (67)     | 11.4±6.5  | 2.8±1.0*  | 1.1±1.1   |
| Control Female (101) | 10.9±4.2  | 1.8±1.2   | 1.3±0.7   |
| IHA (+) Female (134) | 10.5±5.4  | 2.0±0.9   | 1.1±0.7   |
| Total Control (160)  | 10.8±5.8  | 1.9±1.1   | 1.2±0.7   |
| Total IHA (+) (201)  | 11.3±4.0  | 2.8±1.3*  | 1.0±0.6   |

\*Significantly different from control group (p<0.05).

#### 3.3. RSH Levels

Serum RSH levels were found 328 ± 149 µmol/L, 327 ± 181 µmol/L in the control male and seropositive group, respectively. In control female and seropositive male group were 338±148 µmol/L and 340±131 µmol/L,

respectively. In addition, total control and seropositive control group RSH levels were 335±152 µmol/L and 336 ± 172 µmol/L, respectively. Differences between serum RSH levels were not statistically significant in all groups (p> 0.05) (Table 4).

Table 4. RSH levels of patients Cystic Echinococcosis

| PATIENTS                  | RSH ( $\mu\text{mol/L}$ ) |
|---------------------------|---------------------------|
| Control Male (59)         | 328 $\pm$ 149             |
| Seropositive Male (67)    | 327 $\pm$ 181             |
| Control Female (101)      | 388 $\pm$ 143             |
| Seropositive Female (134) | 340 $\pm$ 131             |
| Total Control(160)        | 335 $\pm$ 152             |
| Total Seropositive (201)  | 336 $\pm$ 172             |

\*Significantly different from control group ( $p < 0.05$ ).

### 3.4. MPO Activity

MPO activity was determined increasing both seropositive female and seropositive male when compared with control groups (seropositive female,  $6.1 \pm 2.8$  U/mg; control female,  $3.1 \pm 2.2$  U/mg; seropositive

male,  $7.8 \pm 3.8$  u/mg; control male,  $3.3 \pm 2.2$  U/mg). In addition, there was a significant increase MPO activity in the total seropositive patients (total seropositive,  $6.0 \pm 3.8$  U/mg; total control  $3.1 \pm 2.2$  U/mg). For these results, MPO activities increased in the patients with CE (Table 5).

Table 5. MPO activity of patients Cystic Echinococcosis

| PATIENTS                  | MPO (U/mg.protein) |
|---------------------------|--------------------|
| Control Male (59)         | 3.3 $\pm$ 2.2      |
| Seropositive Male (67)    | 7.8 $\pm$ 3.8*     |
| Control Female (101)      | 3.1 $\pm$ 2.2      |
| Seropositive Female (134) | 6.1 $\pm$ 2.8*     |
| Total Control(160)        | 3.1 $\pm$ 2.2      |
| Total Seropositive (201)  | 6.0 $\pm$ 3.8*     |

\*Significantly different from control group ( $p < 0.05$ ).

## 4. DISCUSSION

CE is an endemic problem that is seen in animals and human in Turkey. CE affects human and animals' health, work power, labor force and economic values negatively [1]. That's why, it is important to note that the CE studies in the world.

The clinical characteristics of the CE and the weak immune response to parasite antigens have caused the appearance of many serological techniques such as ELISA, immunoblotting, immunoelectrophoresis and indirect fluorescent antibody test. Force et al reported that IHA was one of the most sensitive techniques for the preoperative diagnosis of the CE. The specificity for the IHA test was reported as 83-100 % [10, 25]. The results of the specificity of our test (84 % for IHA test) were consistent with the literature.

In our study, we found the IHA test was positive in 201 (55.7%) patients with a titer of 1/160 or higher. The highest titre 1/1280 (46.3%) was found in all patients sera. Females showed a significantly higher seropositivity rate than males in IHA test.

CE may affect several organs in the human body and thus represents a major challenge for the general surgeon. The liver is the most common site for hydatid disease (75% of cases), followed by the lungs (15%), the spleen (5%) [26] and other organs (5%) [27-29] being in accordance with other studies [30-32]. This study were involved

organ was the liver 68.18%, followed by the lung 13.63% and in other organs in the remaining patients.

Immunodiagnosis is an important tool for diagnosis of CE infection. Thus, in addition to imaging techniques, a reliable serodiagnosis improves prognosis for patients, because medical treatment can then be specifically adapted to the CE problem.

Richard demonstrated that IgG levels were found high values in a long period in the hydatid patient's sera by using latex agglutination (LA) and immunoelectrophoresis (IEP) methods [33]. In addition, Force et al (1992) reported that ELISA IgG was the most sensitive (91 %) test when compared to the ELISA IgA, IgE, IHA and immunoelectrophoresis [34]. Another research, IgG and IgM were found to be the major class of serum antibody involved in Turkana and UK hydatid patients [11]. Bulut et al (2001) reported a significant increase of IgA and total IgG levels were determined in the patients with hydatidosis when compared to the healthy volunteers [35]. However, Barış and coworkers (1989) has been showed IgA major antibody in fistula growth lung hydatid cyst patients [13]. In our study, we found IgA levels were determined increasing in the seropositive group when compared to the control. In addition, IgA levels increased male of seropositive patients' sera. However, IgG and IgM levels were not change with seropositive patients with CE.

Chronic inflammation is believed to trigger the cascade of oxidative stress response in the affected organs and tissues. Increased oxidative stress in living systems was scavenged by enzymatic and non-enzymatic antioxidants. Superoxide dismutase (SOD) plays an important role in detoxication of the organism and its function is to protect the organism against the cytotoxic action of free radicals. Kozłowska and Rzymowska (2006) reported that the expression of SOD gene increased at mRNA level in hepatocytes in infection with *E. granulosus* [36]. It has been known that administration of alpha-tocopherol (only a single injection) to patients with hydatid disease of the liver in preoperative period beneficial effect on the decreasing lipid peroxidation [18]. In our study, there was no statistical changes were found in seropositive patients in serum total sulphydryl group levels. This result suggested that other antioxidants may play vital role to scavenge to increased oxidative stress in CE with patients.

There were several studies on host oxidative stress and antioxidant defense mechanism in parasite disease, but

there wasn't any study on researches of RSH levels and MPO activity in patients with CE.

MPO serum levels reflect the ongoing disease and are related to functional activity of the respective leukopoetic system. It has been known that serum MPO activity were altered helminthes infection by increased neutrophil and/or monocyte/macrophage effectors system [37]. In our study, we found increased serum MPO activity both female and male with CE. This result reflects functional activity of the respective leukopoetic system at host defense systems to deal with helminthes reactions.

In conclusions, this study is the first investigation on serum antioxidant levels and MPO activities with CE patients. Further studies will focus on oxidative stress and antioxidant capacities in preoperative and postoperative CE patients.

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