Investigation of Direct Microscopy and Indirect Hemagglutination Test Results in the Diagnosis of *Echinococcus granulosus* in Selçuk University Hospital

Salih Macin, Rugiya Samadzade

Selcuk University, Faculty of Medicine, Department of Medical Microbiology, Konya, Turkey

**ABSTRACT**

**Objective:** Cystic echinococcosis (CE) is a zoonotic disease in humans and herbivorous animals caused by cestodes of the genus *Echinococcus*. Humans are incidental hosts in the infection caused by *E. granulosus*. The aim of this study is to retrospectively investigate the direct microscopy and seropositivity of patients diagnosed with CE.

**Materials and Methods:** Between January 2017 and January 2021, cyst fluid and serum samples from 55 patients were sent to the Parasitology Laboratory from various units of the hospital. Cyst fluid samples were examined microscopically after condensation. Specimens showing hooks or protoscolex on microscopy have been reported as positive. Serum samples of the patients were investigated for cystic echinococcosis by indirect hemagglutination test (IHA). Those with a titer of ≥160 were considered positive.

**Results:** Twenty two (40%) cyst specimens with hooks or protoscolex on direct microscopy were reported as positive. Of the patients included in the study, 38 (69.1%) were male and 17 (30.9%) were female. Both direct microscopy and IHA test were studied from 30 patients. In terms of CE, the serum of 17 (56.6%) patients was found to be seropositive. In addition, both direct microscopy and IHA results of 8 (36.3%) patients were found to be positive.

**Conclusion:** Although CE is quite common in Turkey, it is highly neglected because it usually does not cause symptoms for years and is not reported frequently even though it is mandatory. For this reason, since the clinical findings of CE, which is still an important public health problem in our region, can be confused with other system pathologies, it would be useful to evaluate clinical, radiological, serological and biochemical findings together in the diagnosis.

**Keywords:** Cystic echinococcosis, *Echinococcus granulosus*, Indirect Hemagglutination Test, protoscolex, direct microscopy
hands and drinking water, or by consumption of raw vegetables and fruits (2).

E. granulosus can be involved in any organ at random. CE, which is not encountered in most of the developed countries around the world, is frequently seen in developing countries (3). CE, is a common disease in both animals and humans in Turkey, it also causes significant economic loss. The economic loss caused by hydatid cyst in our country are mainly due to the expenses of diagnosis and treatment of the disease in humans. In farm animals, depending on the spread in the organs during the development of the cyst; factors such as low wool quality, increase in sterility rate, decrease in meat and milk yield are the most important detrimental effects (4).

Since the clinical findings are nonspecific, radiological and serological diagnostic methods are used in the diagnosis of the disease. Radiologic diagnostic methods such as USG, CT and MRI are frequently used in the follow-up of patients with cyst rupture and in the postoperative period. In such cases, serological methods are very useful both in confirming the diagnosis and in the follow-up of postoperative recurrences (5). Most of these methods are based on the detection of antibodies specific to E. granulosus in patient’s sera. ELISA and indirect hemagglutination (IHA) methods are frequently preferred for diagnosis because of the ease in application, low cost, and high sensitivity and specificity (6).

In the microscopic examination of the cyst fluid, seeing the protoscolex of the parasite or even one hook belonging to the protoscolex is sufficient for a definitive diagnosis. Some of the ruptured cysts can be seen in the bronchi, intestine, urinary and biliary tract. Therefore, protoscoleses, hooks and germinal membrane particles of the parasite can be seen in samples taken from these regions (7). However, the absence of typical structures in microscopic examination does not rule out the disease. Because some cysts may be sterile (they may not contain any scolex and daughter cysts) (8).

The aim of this study was to retrospectively investigate the correlation of direct microscopy and seropositivity of patients diagnosed with cystic echinococcosis. In addition, it was aimed to investigate the distribution of antibodies positivity of E. granulosus according to hospital units and gender.

MATERIALS AND METHODS

The results of direct microscopic examination of cyst fluid samples sent to the Medical Microbiology Laboratory from various clinics of Selçuk University Medical Faculty Hospital between January 01, 2017 and January 01, 2021 were examined. In case of protoscolex in cyst fluids, it was centrifuged at 4000 rpm for 15 minutes in order to precipitate. Macroscopically, after centrifugation, a sedimentation or turbidity was detected at the bottom of the liquids, which are protoscoleses. After centrifugation, 1-3 cc droplets were dropped on the slide with a pipette from the sediment, and covered with a coverslip. The preparation was examined under a microscope with 40 objective. Specimens showing hooks and/or protoscolex on microscopy have been reported as positive.

Serum samples of the patients had been investigated for cystic echinococcosis by IHA. Blood had been withdrawn from the patients in 10 cc vacuum tubes with yellow caps. The sera separated by centrifugation for 10 minutes at 4000 rpm, (xg) were used for the study. The IHA reagent is diluted with 2.5 µl of deionized water and vortexed. On 175 µl buffer was placed in the well and 25 µl patient serum was added in the well. Then 50 µl buffer was placed in 2nd, 3rd, 4th, 5th, 6th, 7th wells and diluted by 50 µl dilutions. Finally, 25 µl antigen is placed on it and shaken for 20 seconds. It was incubated for 2 hours. Those with a titer of ≥160 were considered positive. The results were analyzed retrospectively through hospital automation.

Ethical Approval

Blood samples sent for routine microbiological examinations from patients with pre-diagnosis of hydatid cyst were included in this study. Voluntary consent form was read and signed by the people whose sample was used. The study protocol followed ethical guidelines of the Declaration of Helsinki.

RESULTS

Fifty-five cyst fluid samples were examined in our laboratory in a three-year period. Hooks and/or protoscoleses was observed in direct microscopic examination of 22 (40%) of them. Of the cyst fluids examined microscopically, 52 (94.5%) were defined as liver and 3 (5.5%) as lung samples. In addition, 38 (69.1%) of the patients studied for CE were male and 17 (30.9%) were female (Figure 1).

Figure 1. Protoscoleses detected on direct microscopy of cyst fluid

IHA test was studied in the sera of 30 patients from whom direct microscopy had been requested. It was determined that the serum of 17 (56.6%) patients was seropositive...
for CE. The IHA test was also studied in 12 (54.5%) of the
patients with positive direct microscopy. According to the
results, although the number of patients was small, the
sensitivity of both direct microscopy and IHA was found
to be high in 8 (66.6%) patients (Figure 2).

In addition, cyst samples were sent mostly from General
Surgery (47.2%) and Radiology (35.4%) units.

![Distribution of IHA results in serum samples](image)

Figure 2. Distribution of IHA results in serum samples

<table>
<thead>
<tr>
<th>Hospital Units</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Surgery</td>
<td>47.2</td>
</tr>
<tr>
<td>Radiology</td>
<td>35.4</td>
</tr>
<tr>
<td>Pediatric surgery</td>
<td>7.8</td>
</tr>
<tr>
<td>Thoracic surgery</td>
<td>5.5</td>
</tr>
<tr>
<td>Others*</td>
<td></td>
</tr>
<tr>
<td>Gastroenterology, Neurosurgery, Pediatric Gastroenterology and Hepatology</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1: Distribution of cyst samples according to clinics**

**DISCUSSION**

CE is an important zoonotic parasitic infection that threatens public health all over the world. CE spreads widely in Turkey due to reasons such as zoogeographic differences, climatic conditions, lack of education about the disease and transmission routes in the society. It is stated that the prevalence of CE in Turkey is 50 per 100,000 and its incidence is around 2 per 100,000 (9, 10). Serum of suspected CE patients sent to the National Parasitology Reference Laboratories of the Public Health Institution of Turkey as a result of the investigation of anti-*E.granulosus* antibodies in various serological methods in the samples, it was determined that KE still continues to be an important public health problem, although it is decreasing gradually in Ankara and its surroundings (11).

Possanti et al. evaluated 1367 articles on potential risk factors in a systematic review and meta-analysis study investigating the relationship between CE and risk factors in humans, and selected 37 studies including case-control and cross-sectional studies as meta-analysis. As a result of the study, they stated that the biggest risks statistically, were stray dogs living in endemic areas, reaching offal, owning a dog and keeping a dog in their homes. However, they also emphasized in their research that the risk factors will differ between geographically different regions and communities (12).

In order to determine positivity of CE in Konya region, many studies have been carried out using the IHA method. Başer et al. found that 332 (21.6%) of 1543 patient samples were seropositive for CE using the IHA method (13). Tasbent et al. seropositivity was detected in 143 (15.2%) of 938 patients included in the study by IHA test (14). In our study, positivity was found to be higher because only specific patients who underwent direct microscopy were included (56.6%). For this reason, similar studies conducted in Konya show that CE still remains as one of the important health problems in our region.

The absence of specific diagnostic clinical findings in human CE has led to the use of laboratory findings rather than clinical findings in the diagnosis of the disease. On the other hand, the preference of surgical treatment due to the inadequacy of medical treatment in the treatment of the disease and the possibility that CE surgery may bring some complications increase the importance of diagnosis in CE (15). Radiological diagnostic methods have an important place in the diagnosis of CE. However, radiological diagnosis should be supported by serological diagnostic methods in order to make the differential diagnosis of cyst with other space-occupying cases such as tumor, abscess, and simple cyst and to evaluate recurrences after the operation in a healthier way (16).

IHA was first used in the diagnosis of CE in 1957 by Garabedian et al. and it was found positive in 13 (81%) of 16 patients (17). Although the sensitivity of the test generally varies between 80-94%, some researchers found sensitivity values as low as 54-65%. The specificity of the test varies between 92-100%. It is known that the sensitivity of the antibody response and serological tests


