

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

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ABSTRACT

Objective: Cystic echinococcosis (CE) is a zoonotic disease in humans and herbivorous animals caused by cestods of the genus *Echinococcus*. Humans are incidental hosts in the infection caused by *Echinococcus 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

INTRODUCTION

Cystic echinococcosis (CE), also known as Hydatid Cyst Disease, is a zoonotic infection caused by *Echinococcus granulosus* (*E. granulosus*), a cestode species. The adult parasite lives in the breed of dogs (dog, fox, wolf). It

infects animals such as sheep, goats and cattle, which are natural intermediate hosts, through its larval form in the metacystode period (1). Humans are incidental hosts in the infection caused by *E. granulosus*. CE disease develops as a result of the ingestion of eggs excreted with the feces of carnivores, which are definitive hosts, with contaminated

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, protoscoleces, 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 protoscoleces. 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 protoscoleces 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).

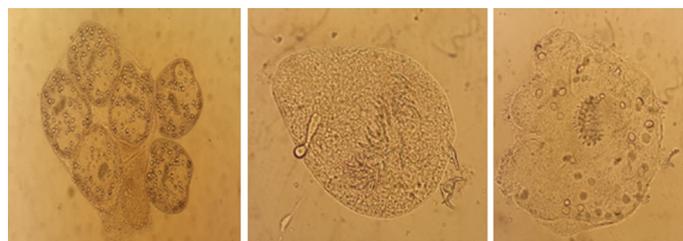


Figure 1. Protoscoleces detected on direct microscopy of cyst fluid

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.

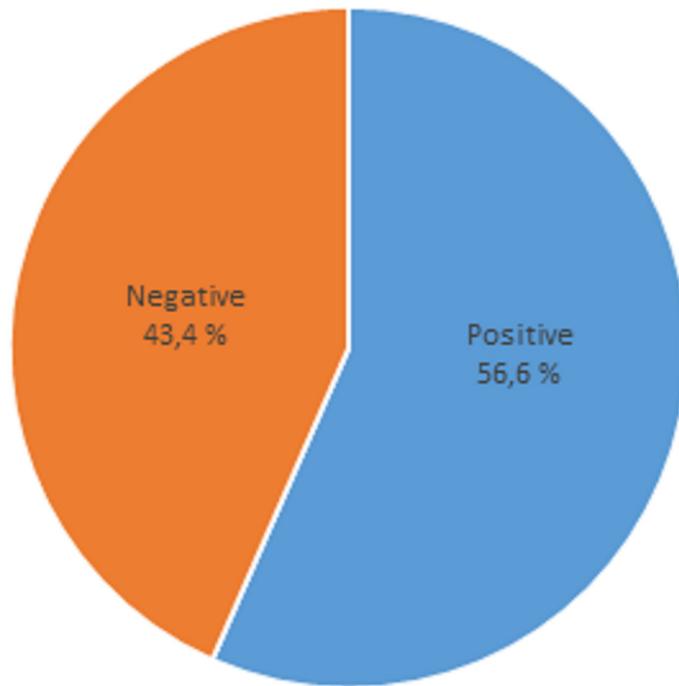


Figure 2. Distribution of IHA results in serum samples

Hospital Units	%
General Surgery	47.2
Radiology	35.4
Pediatric surgery	7.8
Thoracic surgery	5.5
Others* Gastroenterology, Neurosurgery, Pediatric Gastroenterology and Hepatology	

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

varies according to the localization of the cyst. Some researchers found the IHA test positivity in 73% of lung cysts and 89% of liver cysts. Other researchers, on the other hand, found IHA positivity in 59% of lung cysts and 76% of liver cysts (18).

Toraman et al. investigated anti-*E.granulosus* IgG antibodies by IHA method in the serum samples with the suspicion of CE. According to the results, seropositivity was found in 12.9% of 162 patients at dilutions of 1/160 and above (19).

Presence of immunocomplexes may also cause low seropositivity with IHA. Many researchers focusing on false positivities in the test attribute them to the type and preparation of the antigen used. In other studies, it was associated with cross-reactions between the aforementioned disease antigens and serum antibodies against *E.granulosus* in low serum dilutions of people with diseases such as taeniosis, fascioliosis, schistomiosis, cysticercosis, liver cirrhosis, and malignancy (20).

Studies have shown that the lack of a test that can definitively detect the presence of hydatid cyst, which has a complex antigenic structure, causes different seropositivity and seronegativity in terms of diagnosis in different patient groups (21). Kaya et al. examined blood samples of 314 people in the settlements within a 5 km area around the slaughterhouses, for *E.granulosus* antibodies by ELISA and IHA methods (22). The study was planned based on the high number of dogs around the slaughterhouses. As a result of ELISA test, 11 people (3.50%) and as a result of the IHA test, 12 people (3.82%) were seropositive. Both ELISA and IHA tests were positive in 9 patients (2.8%). Artun et al. analyzed serum samples of 100 patients with pre-diagnosis of hydatid cyst using IgG-ELISA, IHA and Western blot methods. As a result, specific antibodies were detected in 20 patients with IgG-ELISA, 16 patients with IHA and 31 patients with Western Blot (18).

There are publications reporting that the incidence of CE is higher in women than in men. In addition, there are also studies showing CE at equal rates in men and women (23). It is thought that this difference between studies may be due to women working more in rural areas and dealing with animals more in some regions (24). In our study, because you only examined the results of patients with pre-diagnosis, it supports that more men than women admitted to the hospital, and that the disease is more common in men than in women.

CONCLUSION

CE is still a neglected zoonotic disease that affects humans socio-economically, has high morbidity and mortality rates, and causes serious economic losses. CE is one of

the important helminth diseases in our country, and the data were mostly obtained from hospitals, and there are a limited number of population-based epidemiological studies. The disease continue to be an important public health problem in our region, especially since the Central Anatolia region and the vicinity of Konya are the places where agriculture and animal husbandry is intense. For this reason, advanced techniques such as serological methods, computed tomography and magnetic resonance imaging are useful in confirming the diagnosis and in preparation for surgical intervention. In addition, it was concluded that the IHA or ELISA method should be preferred in the routine laboratory diagnosis of CE and the results should be confirmed with WB if possible.

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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.

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