Patients with Cystic Echinococcosis: Evaluation of Clinical and Biological Features of Cysts

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ABSTRACT

Objective: The main purpose of the study was to evaluate CE patients in a versatile way with the most commonly used diagnostic methods. In addition, we also investigated correlation between diagnostic parameters and CE cyst properties.

Materials and Methods: A total 45 patients had an active or transitional CE cysts according to the WHO-IWGE classification and they have received percutaneous treatment for CE cysts. Serological analyses, molecular characterization of E.granulosus s.l. and direct examination of hydatid material were performed.

Results: A total of 58 CE cysts were recorded and 34 of 58 hydatid cysts were identified as CE1, 14 as CE2, 2 as CE3a and 8 as CE3b. Direct examination, 30 of 58 hydatid cysts were found to be positive regarding protoscolex. According to serological tests, 37 of 45 patients had positive ELISA results. Most of the isolates (56/58) were identified as E.granulosus sensu stricto (G1- G3 genotypes) and two of them were characterized as E. canadensis (G6/7 genotypes). On the other hand, it is confirmed that serodiagnosis has been influenced by certain cyst characteristics.

Conclusion: CE patients should be evaluated using many diagnostic approaches to accurate epidemiological information and to adopt proper clinical management.

Keywords: Cystic echinococcosis, protoscolex, serodiagnosis, Echinococcus granulosus, hydatid cyst

INTRODUCTION

Cystic echinococcosis (CE) is a neglected zoonotic disease caused by metacestode form of Echinococcus granulosus sensu lato. The life cycle of E. granulosus s.l. mainly perpetuates between canids and livestock animals especially sheep and cattle. Humans are dead-end intermediate hosts that accidentally infect with parasite eggs (1). Molecular analyses have shown that E. granulosus s.l. includes at least 4 species as Echinococcus granulosus sensu stricto (s.s.)(G1 and G3 genotypes), Echinococcusequinus (G4 genotype), Echinococcus ortleppi (G5 genotype) and Echinococcus canadensis cluster (G6/7, G8, G10 genotypes) (2-5). E. granulosus s.s. , has a worldwide distribution and it
is responsible for the majority of cases (88.44%) followed by *E. canadensis* (G6/G7) (11.07%), *E. ortleppi* (G5) (0.36%), *E. canadensis* (G8) (0.06%) and *E. canadensis* (G10) (0.06%) (6). CE is a cosmopolitan disease that is more common in rural areas. According to a population-based screening study, over 100,000 people living in pastoral areas of Turkey might be suffered from abdominal CE (7). In a recent report, the number of cases substantially increased between 2008-2019 in Turkey. Although the increase in the number of registered cases is due to the revision of the surveillance system of the Ministry of Health, there are considerable cases of CE in the region (8).

The World Health Organization (WHO) has recently announced a road map for neglected diseases including Echinococcosis targeted for prevention, control, elimination, and eradication by 2030 (9). According to the document, there is a need to define target product profile and develop optimal diagnostic for humans. As known, CE diagnosis is mainly based on imaging modalities and it is sometimes difficult even in a fully equipped health facility (10). Although CE cysts can be located in every organ, the liver is the most involved organ with a 70% rate (11). The WHO-IWGE (World Health Organization-Informal Working Group on Echinococcosis) has made widely accepted classification for CE cysts. According to this classification, CE cysts can be classified as active (CE1 and CE2), transitional (CE3a/CE3b) and inactive (CE4 and CE5) (12). Whereas imaging techniques are the primary tool for the CE diagnosis, serological tests have an auxiliary role even though these are not standardized for CE (10). Direct microscopic examination of aspirated cyst fluid or DNA detection with polymerase chain reaction (PCR) technique from hydatid material can be used as a confirmatory tools for CE diagnosis. Additionally, PCR-based methods show high specificity and sensitivity rates, as well as useful for the characterization of the species, and genotypes (13).

The main purpose of the study was to evaluate CE patients in a versatile way with the most commonly used diagnostic methods and with the contribution of well-defined and confirmed 45 CE patients.

**MATERIALS AND METHODS**

**Ethical considerations**

This study was approved by the Local Ethical Committee (Ethical Committee of the Faculty of Medicine, Hacettepe University, Turkey 2018; GO 18/366-15).

**Sample Collection**

Sample collection was carried out from May to November 2018. All the patients had an active or transitional CE cysts (CE1, CE2, CE3a and CE3b) according to the WHO-IWGE classification and they have received percutaneous treatment for CE cysts. Patients with inactive CE cysts were not included in the study. Blood samples were taken from patients at the first diagnosis, therefore any medication (e.g. albendazole) was not used by patients before sample collection. In addition, hydatid material was collected during the procedure.

**Serological Tests**

All the collected sera were stored at -80°C until they were tested. Obtained sera were evaluated using the following commercially available serological test: Hydatidosis IgG ELISA (Vircell SL, Granada, Spain) according to manufacturer’s instruction. Sample Index (SI) which is calculated using Optical Density (OD) was used to interpret the ELISA results according to manufacturer’s recommendation. Accordingly, ELISA results were accepted negative for SI <0.9, positive for SI 1.1 and border line for 0.9 ≤SI<1.1. Borderline results were accepted negative. All results were reported as positive or negative. All tests were performed in a same session.

**Direct Examination of Hydatid Material**

Aspirated cyst fluid was transferred to a new sterile tube, and after centrifugation at 3000g for 3 minutes, the precipitate was examined by light microscope for the presence of protoscoleces or hooks.

**Genetic Characterization of *E.granulosus s.l.* and Data Analysis**

The DNA extraction from hydatid material was performed using GeneMATRIX Universal DNA/RNA/Protein Purification Kit (EURx, Poland) according to manufacturer’s instructions. The PCR was performed to amplify a fragment within the cytochrome c oxidase subunit 1 (cox 1) mitochondrial gene, as previously reported (14). Electrophoresis in 1.5% agarose gels was used to visualize PCR products under ultraviolet light. The amplicons were evaluated as positive if a band size of ~875 bp was obtained. All products were identified by sequencing. Obtained sequence data was analyzed via FinchTV 1.4.0 (Geospiza Inc., Seattle Washington, USA). The BLAST database (http://www.ncbi.nlm.nih.gov/BLAST/) was used to find and compare the homologous sequences in the GenBank. For data analysis, sequence alignment was performed in Mega version 7 (15) and ClustalX (16).

IBM SPSS Statistics program Ver. 23 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The chi-square test was performed for categorical data.

**RESULTS**

Forty-five patients (23 female and 22 male, mean age: 34.9) were included in this study. At the first diagnosis, 37 of 45 patients (82%) had positive ELISA results. Among the ELISA-negative patients, cyst material belonging to five of them was found negative in the direct examination, as well. In patients with a positive ELISA result (33/37, 89%), almost all of their cysts were found in the liver. Features of patients and hydatid cysts are given in Table 1.
| Patient No | Sex     | Number | Type  | Location | Diameter (cm) | Volume (cm³) | Protoscolex | ELISA   | Species              |
|------------|---------|--------|-------|----------|---------------|--------------|-------------|---------|----------------------|
| 1          | Male    | 1      | CE1   | Liver    | <5            | 27.2         | Negative    | Negative | E.granulosus s.s.   |
| 2          | Male    | 1      | CE1   | Spleen   | <5            | 57.5         | Negative    | Positive | E.canadensis         |
| 3          | Female  | Multipl| CE1   | Liver    | 5-10          | 304.5        | Positive    | Positive | E.granulosus s.s.   |
| 4          | Male    | 1      | CE1   | Kidney   | <5            | 21.84        | Negative    | Negative | E.granulosus s.s.   |
| 5          | Male    | Multipl| CE1   | Liver    | >10           | 471.9        | Positive    | Positive | E.granulosus s.s.   |
| 6          | Female  | 1      | CE1   | Liver    | <5            | 20           | Negative    | Positive | E.granulosus s.s.   |
| 7          | Male    | 1      | CE1   | Liver    | >10           | 423.5        | Negative    | Positive | E.granulosus s.s.   |
| 8          | Male    | 1      | CE1   | Liver    | 5-10          | 170.54       | Negative    | Positive | E.granulosus s.s.   |
| 9          | Male    | 1      | CE1   | Liver    | 5-10          | 315          | Positive    | Positive | E.granulosus s.s.   |
| 10         | Female  | 1      | CE1   | Liver    | 5-10          | 196.8        | Negative    | Positive | E.granulosus s.s.   |
| 11         | Female  | Multipl| CE1   | Liver    | 5-10          | 49.5         | Negative    | Positive | E.granulosus s.s.   |
| 12         | Male    | 1      | CE1   | Liver    | 5-10          | 405          | Positive    | Positive | E.granulosus s.s.   |
| 13         | Female  | 1      | CE1   | Spleen   | <5            | 50           | Negative    | Positive | E.granulosus s.s.   |
| 14         | Female  | 1      | CE1   | Liver    | >10           | 577.5        | Positive    | Positive | E.granulosus s.s.   |
| 15         | Male    | Multipl| CE1   | Liver    | 5-10          | 324          | Positive    | Positive | E.granulosus s.s.   |
| 16         | Female  | 1      | CE1   | Liver    | 5-10          | 159.7        | Negative    | Positive | E.granulosus s.s.   |
| 17         | Female  | 1      | CE1   | Liver    | 5-10          | 109.54       | Positive    | Positive | E.granulosus s.s.   |
| 18         | Male    | 1      | CE1   | Liver    | 5-10          | 105.17       | Positive    | Positive | E.granulosus s.s.   |
| 19         | Female  | 1      | CE1   | Liver    | >10           | 875          | Negative    | Positive | E.granulosus s.s.   |
| 20         | Male    | 1      | CE1   | Liver    | 5-10          | 70           | Positive    | Negative | E.granulosus s.s.   |
| 21         | Female  | 1      | CE1   | Liver    | 5-10          | 556.76       | Negative    | Positive | E.granulosus s.s.   |
| 22         | Female  | 1      | CE1   | Liver    | 5-10          | 138.62       | Positive    | Positive | E.granulosus s.s.   |
| 23         | Male    | 1      | CE1   | Liver    | >10           | 759          | Positive    | Positive | E.granulosus s.s.   |
| 24         | Male    | 1      | CE1   | Liver    | >10           | 208          | Negative    | Positive | E.granulosus s.s.   |
| 25         | Male    | Multipl| CE1   | Liver    | >10           | 598.78       | Positive    | Positive | E.granulosus s.s.   |
| 26         | Male    | Multipl| CE1   | Spleen   | >10           | 996.87       | Positive    | Positive | E.granulosus s.s.   |
| 27         | Male    | Multipl| CE1   | Liver    | 5-10          | 252          | Negative    | Positive | E.granulosus s.s.   |
| 28         | Female  | Multipl| CE1   | Liver    | >10           | 1274         | Positive    | Positive | E.granulosus s.s.   |
| 29         | Male    | 1      | CE2   | Kidney   | 5-10          | 383.56       | Positive    | Negative | E.granulosus s.s.   |
| 30         | Male    | 1      | CE2   | Liver    | 5-10          | 275.26       | Positive    | Positive | E.granulosus s.s.   |
| 31         | Male    | Multipl| CE2   | Liver    | <5            | 62.19        | Negative    | Positive | E.granulosus s.s.   |
| 32         | Female  | Multipl| CE2   | Liver    | 5-10          | 165.75       | Negative    | Positive | E.granulosus s.s.   |
| 33         | Female  | Multipl| CE2   | Liver    | <5            | 54.77        | Negative    | Positive | E.granulosus s.s.   |
| 34         | Female  | 1      | CE2   | Bone     | 5-10          | 101          | Positive    | Negative | E.canadensis         |
| 35         | Female  | 1      | CE2   | Kidney   | >10           | 1013         | Positive    | Positive | E.granulosus s.s.   |
| 36         | Male    | 1      | CE2   | Liver    | 5-10          | 160          | Positive    | Positive | E.granulosus s.s.   |
| 37         | Male    | 1      | CE2   | Liver    | 5-10          | 72           | Negative    | Negative | E.granulosus s.s.   |
| 38         | Female  | Multipl| CE3a  | Liver    | <5            | 50           | Negative    | Positive | E.granulosus s.s.   |
| 39         | Female  | 1      | CE3b  | Liver    | 5-10          | 129          | Negative    | Negative | E.granulosus s.s.   |
| 40         | Female  | 1      | CE3b  | Liver    | >10           | 389.88       | Negative    | Positive | E.granulosus s.s.   |
| 41         | Female  | 1      | CE3b  | Liver    | 5-10          | 192          | Positive    | Positive | E.granulosus s.s.   |
| 42         | Female  | 1      | CE3b  | Liver    | 5-10          | 219.37       | Negative    | Positive | E.granulosus s.s.   |
| 43         | Female  | 1      | CE3b  | Liver    | 5-10          | 92.25        | Negative    | Positive | E.granulosus s.s.   |
| 44         | Male    | 1      | CE3b  | Liver    | 5-10          | 210.9        | Positive    | Positive | E.granulosus s.s.   |
| 45         | Female  | Multipl| CE3b  | Liver    | <5            | 13.86        | Negative    | Negative | E.granulosus s.s.   |
A total of 58 CE cysts were recorded and cyst type were determined based on WHO-IWGE classification. According to this, 34 of 58 hydatid cysts were classified as CE1, 14 as CE2, 2 as CE3a and 8 as CE3b. The hydatid cysts were predominantly located in the liver (51/58) and also were detected in the spleen (3/58), kidney (3/58), and bone (1/58). The average diameter of hydatid cysts was found to be 8.25 cm.

Of the 58 cyst samples were examined for the presence of protoscolex, 30 of them (51%) were found to be positive and 28 (49%) were negative. There was no relationship between presence of protoscolex and patient characteristics.

All the isolates were confirmed by the BLAST algorithm as E. granulosus s.l. Almost all isolates (56/58) were identified as E. granulosus s.s. (G1/G3 cluster) and only two of isolates belonged to E. canadensis (G6/7 cluster). Due to the small number of samples belonging to E. canadensis, we were not able to investigate the relationship between genetic diversity and CE related-parameters.

The relationship between serodiagnosis results and cyst characteristics such as type, number, location and diameter were also investigated. There was no relationship between serological test results and cyst type and cyst number. On the other hand, positive serodiagnosis result was significantly associated with cyst size (p=0.018) and cyst location (p=0.027).

**DISCUSSION**

Although the prognosis of CE predominantly proceeds asymptomatic, management of CE depends on multiple factors (e.g., cyst number and stage, cyst location, etc.). Due to this multifactor process, diagnosis and follow-up of patients are still problematic, due to several drawbacks related to the available supporting methods used to complement the imaging findings (13). Integrating many different methods for the diagnosis of CE provides more accurate results (17). In this study, forty-five well-defined CE patients were presented with details of diagnosis.

In terms of protoscolex presence, all hydatid material was evaluated and almost half of them found negative. Although direct examination of cysts is one of the confirmatory tools for diagnosis, molecular techniques such as PCR should be performed for negative samples (18). In accordance with this view, all hydatid cysts (with or without protoscolex) were confirmed molecularly and identified as E. granulosus s.l in this study. Among the ELISA-negative patients, cyst material belonging to five of them was found negative in the direct examination. Besides, four of these patients had CE cysts lower than 5 cm diameters. Therefore, it is shown that molecular techniques should be used as a confirmatory tool for CE, especially in presence of non-specific imaging findings.

Many studies have indicated that E. granulosus s.l. show high genetic diversity (19). In addition, several study were conducted to define the relationship between genetic diversity and cyst characteristics. As an example, cerebral CE was found to be associated with the G6 genotype (E. canadensis) (20). According to a retrospective study, cyst size was significantly small in G7 genotype (E. canadensis) compared to G1 genotype (E. granulosus s.s.) (21). In a recent study, cyst volume was found to be related with genetic diversity (22). In the present study, the relationship between genetic diversity and cyst characteristics could not be investigated due to the small number of isolates that belonged to E. canadensis (2/58).

As known, serology has a complementary role in the diagnosis of CE and cannot be used to guide without imaging findings for the management of CE. Serology results vary depending on the cyst characteristics such as type, number, size (13). In the present study, the majority of patients (37/45) had positive ELISA results. The relationship between serological test results and other parameters was investigated. As a result, it has been noticed that cyst diameter, as well as cyst volume, influenced serodiagnosis. (p=0.018, p=0.36). Consistent with other studies (23,24), it was noted that as the size of the cyst increased, the serological test results tend to be positive. Besides, all the patients harbored giant hydatid cysts (>10 cm) were found to be serology positive. We have shown that serological test results were influenced also by cyst location (p=0.027). In harmony with our results, most of the studies have supported that serodiagnosis is non-reliable when the cyst involved other than the liver (23). In contrast with other studies, cyst properties such as cyst type, cyst number, and presence of protoscolex were not found to be related to serological test results (23,25-28).

**CONCLUSION**

In conclusion, microbiological confirmation of the radiological diagnosis of CE is critical for accurate epidemiological information. In the present study, we have used different diagnostic approaches of CE and properly compared them. As a consequence, the best tool for CE diagnosis other than imaging techniques is still the subject of debate. This study suggests that even if the absence of protoscolex in direct examination and the serological test results are negative, it will definitely be useful to perform molecular techniques.

**Competing interests:** The authors declare that they have no competing interest.

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**Ethical approval:** This study was approved by the Local
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