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### Case Report

## Clinical and Molecular Evaluation of a Case of Giant Primary Splenic Hydatid Cyst: A Case Report

Bahador SARKARI<sup>1</sup>, Asieh FATEMI ESFEDAN<sup>2</sup>, \*Abdolali MOSHFE<sup>3</sup>, Samaneh ABDOLAHI KHABISI<sup>2</sup>, Amir SAVARDASHTAKI<sup>2</sup>, Farshad HOSSEINI<sup>4</sup>, Ardavan SHAHBAZI<sup>5</sup>

1. Basic Sciences in Infectious Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
2. Dept. of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
3. Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, Iran
4. Dept. of Radiology, Imam Sadjad Hospital, Yasuj, Iran
5. Dept. of Surgery, Shahid Beheshti Hospital, Yasuj, Iran

Received 12 Feb 2016  
Accepted 25 Aug 2016

**Keywords:**  
Primary,  
Splenic,  
Hydatid cyst,  
Iran

**\*Correspondence  
Email:**  
[amoshfea@yahoo.com](mailto:amoshfea@yahoo.com)

### **Abstract**

Spleen is an unusual location for hydatid cyst. Here we report a case of primary splenic hydatid cyst in a 41-yr-old Iranian woman from Yasuj, southwest of Iran. The patient had been admitted to Shahid Beheshti Hospital because of abdominal pain. Abdominal sonography revealed a hypoechoic lesion of 150 X 130 mm in the spleen, suggestive of hydatid cyst. Splenectomy was performed for the patient and surgical interventions revealed a hydatid cyst occupying most of splenic parenchyma. She was discharged on the 5 day of her operation. Postoperative diagnosis and confirmation of hydatid cyst was done by histopathological, molecular and serological approaches. Histopathological evaluation revealed the classical laminated layer of hydatid cyst. DNA was extracted from a part of cyst and PCR amplified. Sequencing and analysis of PCR product revealed that the isolate has the most similarity with G1 strain of *Echinococcus granulosus*. Patient's serum was positive for IgG anti-hydatid cyst antibodies, using antigen-B ELISA.

## Introduction

Cystic echinococcosis (CE) is the larval cystic stage of the tapeworm *Echinococcus granulosus* (1). The disease is endemic in the Central Asia, Mediterranean countries; Middle East, Africa and South America (1-2). Human become infected by accidentally ingesting of contaminated food and water with eggs of the tapeworm (1, 3). Ingestion of eggs of the adult parasite results in the development of one or several unilocular hydatid cysts in humans, mainly in the liver (70%) and lungs (20%). However, the larvae may pass through the liver and lung barriers and reach the other body sites. The unusual locations of hydatid cyst in human are included, but not limited to, the heart, orbit, brain, spleen, muscle, salivary gland, bone, urinary tract, and pancreas (4). Frequency of splenic hydatid cyst is low (about 0.5 to 6% of total incidence of hydatid cyst) even in endemic areas (4, 2).

Splenic hydatid cyst is usually asymptomatic and symptoms are few and nonspecific and patients may be asymptomatic for 5 up to 20 years before the diagnosis (5). Complications with splenic hydatid cyst are including, secondary infection, fistulization to adjacent tissues and cyst rupture to the peritoneal cavity, which in turn may cause a life-threatening systematic anaphylactic reaction.

Primary localization of hydatid cyst in unusual locations is a diagnostic challenge for clinicians. Diagnosis of splenic hydatid cyst is usually established during abdominal ultrasound exam, performed for other clinical reasons.

The biological variants of *E. granulosus* have been designated as strains. Based on mitochondrial DNA (mtDNA) analysis, the *E. granulosus* complex has been split into *E. granulosus sensu stricto* (genotypes G1, G2 and G3), *E. equinus* (G4), *E. ortleppi* (G5) and the still controversial taxon *E. canadensis* (G6-G10)" (6). Most of human cases are related to G1 strain of *E. granulosus*.

Here we report a rare case of primary giant hydatid cyst. The case was evaluated by molecular, histopathological and serological approaches.

## Case

A 41-yr-old woman admitted at department of surgery at Shahid Beheshti Hospital in Yasuj, southwest of Iran. She had been admitted because of abdominal pain. Informed consent was taken from the patient. Abdominal sonography evaluation revealed a large cyst in the spleen, measuring 150 X 130 mm. The cyst had a tick wall protruded from inferomedial aspect of spleen with patches of wall calcification. Preoperative diagnosis, based on cyst characteristics, was splenic hydatid cyst. Low level of hemoglobin (Hb=10.7 grams per deciliter) was noticed in her hematology tests. The rest of biochemistry, and hematological indices were normal. Splenectomy was performed for the patient and surgical interventions revealed a hydatid cyst occupying most of splenic parenchyma (Fig. 1-4).



**Fig. 1:** Gross picture of the spleen hydatid cyst (Original)

She was discharged on 5th postoperative day. Postoperative diagnosis and confirmation of hydatid cyst was done by histopathological, molecular and serological approaches.

Histopathological evaluation revealed the classical laminated layer of hydatid cyst. Patient serum was evaluated by AgB-ELISA, as described before, and was found to be positive by this sensitive serological assay (7).

The patient was housekeeper, used to have contact with animals including dogs and was from a known CE endemic area, where the hydatid cyst is quite common in both human and animals (2).



**Fig. 2:** Cut section of the splenic hydatid cyst with huge secondary infection (Original)



**Fig. 3:** Cut section of the splenic hydatid cyst showing calcified cyst layer (Original)

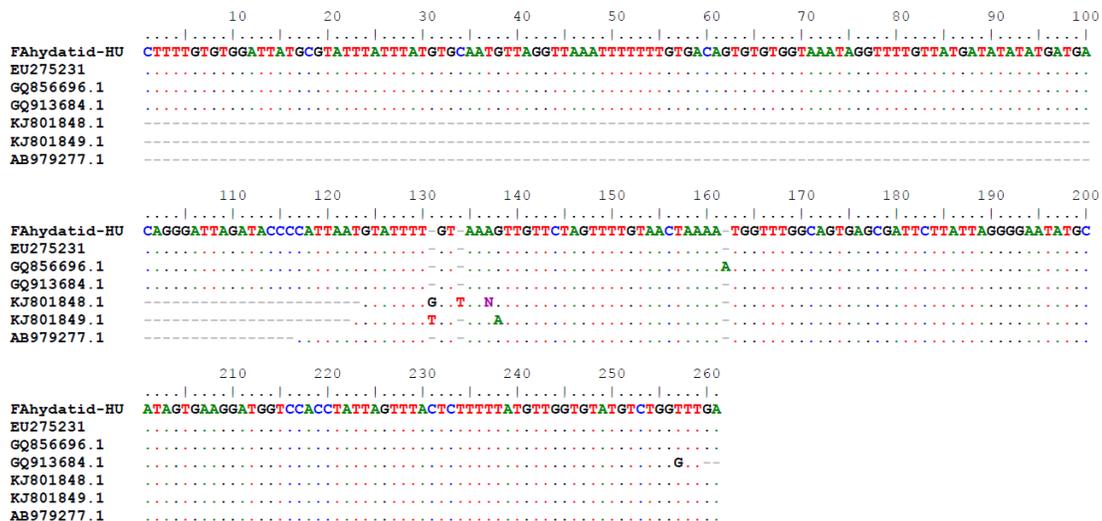


**Fig. 4:** Isolated sections of the cyst showing the cyst layers (Original)

**Molecular evaluation**

For the purpose of molecular characterization, a few parts of the cyst were stored in 70% ethanol until use. DNA was extracted from the sample. Extraction was done using 15 µL of proteinase K and 300 µL of lysis buffer (50 ml of Tris-HCl (100 Mm), pH = 8; 1 mM of EDTA, pH= 8; 1% Tween 20) followed by phenol/chloroform/ethanol/acetate extraction. Absolute ethanol was used to precipitate the DNA. Precipitated DNA was re-suspended in 100 µL of double-distilled water and stored at -20°C until use. PCR primer sets were used for amplifying a 280 bp fragment of mitochondrial 12S rRNA gene of *E. granulosus*, using G1 Forward: 5'–GCTTTTGTGTGGATTATGCG-3' and G1 Reverse: 5'– TCAAACCAGACATACACCAA- 3' primers (8). PCR reaction was carried out in a final volume of 25 µl, containing 2 µL of DNA template, 0.5 µL of each primer (10 Pmol), 12.5 µL of master mix and 9.5 µL of double-distilled water. PCR product was separated by electrophoresis in 1.5% agarose gel and stained with Gel-red. Thermocycler was

programmed by one cycle of initial denaturation at 94 °C for 4min, followed by 35 cycle of denaturation at 94 °C for 94 seconds, annealing at 57 °C for 30 seconds, extension at 72 °C for 35 seconds and final extension at 72 °C for 4 min. After PCR amplification, an approximately 280 bp PCR product was amplified. PCR product was excised from the 1.5% agarose gel and purified with a DNA Gel Extraction Kit, according to the manufacturer's instructions (Bioneer's AccuPrep Gel Purification Kit). PCR product was sequenced and the sequence of the isolate was aligned and compared with those of available sequences of *E. granulosus* in the GenBank. The sequence of the isolate showed the most identity with accessible sequences of *Echinococcus* G1 genotypes, including EU275231.1 (from sheep from Iran), GQ856696.1 (from bovine from Iran), GQ913684.1 (from goat from Iran), KJ801848.1 (from sheep from Argentina), KJ801849.1 (from human from Yemen) and AB979277.1 (From human from Japan) (Fig 5).



**Fig. 5:** Alignment of sequence of the 12S rRNA gene of *E. granulosus* isolated from the patient with some 12S rRNA gene of *E. granulosus* available in the GenBank. FAhydatid-HU: Sequence of the current splenic hydatid cyst; EU275231.1 (from sheep, Iran); GQ856696.1 (from bovine, Iran); GQ913684.1 (from goat, Iran); KJ801848.1 (from sheep, Argentina); KJ801849.1 (from human, Yemen); AB979277.1 (from human, Japan)

## Discussion

Hydatid disease is an endemic zoonosis in many parts of Iran and is an important medical and veterinary concern in many countries. The most common locations for hydatid disease are the liver and lungs, but any organ or tissue can potentially be affected by this disease (4-5). Primary splenic involvement in hydatid cyst is uncommon, as the parasite embryo usually trapped in the liver (as first filter) and the lung (as second filter).

Clinical presentation of hydatid disease depends on the size, site and depth of the cyst. Splenic hydatid cyst is usually slowed growing and asymptomatic. The most common symptoms are abdominal pain and palpable mass in the left upper quadrant. Rapture of spleen cyst to thorax may cause severe spleno-thoracic fistula. Moreover, rapture of spleen cyst may cause systemic anaphylactic reaction leading to dyspnea, pruritus, edema or even death. The majority of splenic infestations are diagnosed following incidental findings at radiographic examination for unrelated complaints.

Hydatid cyst is diagnosed mainly by history, imaging, and serological methods (9-14). The performance of serological tests in the diagnosis of splenic hydatid cyst might not be satisfactory. In a series of 6 cases of splenic hydatid cyst, only 3 cases (concurrent cysts in spleen and liver) were positive by the serological methods and the remaining 3 cases, which had only splenic hydatid cyst, were negative (15). In two cases of splenic hydatid cyst in Greece, both have been positive by an immunoblotting test (16). In our case, serological evaluation revealed anti-hydatid cyst antibodies in patient serum, using antigen-B ELISA. Seropositivity of our case might be contributed to the use of a reliable serological test and the feature of the current splenic cyst, which was large and primary. Blood eosinophilia may present in less than 30% of patients with splenic hydatid cyst, make this blood paramete-

ter an unreliable laboratory finding. Blood eosinophilia was not present in our case. For treatment of splenic hydatid cyst, splenectomy is preferred in adults and is required when the cyst is particularly large or centrally located (17).

So far, ten different genotypes (G1-G10) have been identified for *E. granulosus* sensu lato by molecular approaches (6). The G1 genotype or sheep strain of *E. granulosus* sensu stricto is the most common genotype of *E. granulosus* in animals and humans worldwide (6, 18-19). The predominant strain of *E. granulosus* in human cases of hydatid cyst in Iran is *E. granulosus* sensu stricto (20). In keeping with this, genotype of our case was G1 sensu stricto of *E. granulosus*.

## Conclusion

Although rare, hydatid cyst should be considered in differential diagnosis of all cystic masses in the spleen in any CE-endemic areas.

## Acknowledgments

Technical assistance of Mr. Arefkhah is highly appreciated. The authors declare that there is no conflict of interest.

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