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Original Research Article

Histopathological and Molecular Diagnosis of Eight Clinical Human Hydatidosis from the Gaza Strip, Palestine

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Abstract

Hydatidosis is a parasitic disease caused by the cestode *Echinococcus granulosus*. The present study focused on the multi-diagnosis of a clinical case including; histopathology, the clinical presentation of the patient, and the molecular diagnosis of the tissue. This is a cross-sectional study for patients diagnosed with hydatid cyst disease. Four hospitals in Gaza Strip were included as follows: European Gaza Hospital, AL-Shifa Hospital, Al-Ahli Arab Hospital, and Nasser Hospital (Histopathology Department). A total of 15 clinical cases of hydatidosis are described, with hydatid cysts collected from the four hospitals diagnosed by the clinical presentation of each case, histopathology, and molecular diagnosis. A total of 46.7% of the examined hydatid cyst disease cases were from the liver. The sequencing and analysis revealed one genotype of *E. granulosus* (G1) responsible for these human hydatid cysts. It is concluded that hydatid cyst disease occurrence is confirmed in the examined human tissue samples and belongs to genotype G1. It is recommended that hydatisosis is known among senior surgeons, we recommend that these cases should be presented in front of early career surgeons, interns and Internists in Gaza Strip Hospitals.

Keywords: Hydatisosis, Histopathology, Gaza, cases, Molecular, diagnosis.

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INTRODUCTION

Echinococcus granulosus is a tapeworm and causes cystic echinococcosis which is one of the neglected zoonosis with a massive socio-economic impact [1, 2]. During its life cylce *E. granulosus* needs two mammalian hosts: a definitive host (dogs) in which the adult stage develops and an intermediate host (including sheep, goats and cattle, pigs) in which the hydatid cyst, grows up [3]. Humans can get an infection with *Echinococcus* when ingesting the eggs from an infected dog. The liver and lungs (60% and 30%, respectively) are the most common sites of hydatid cysts; which also affect the kidneys, bones, brain, and pericardium [4]. World Health Organization (WHO) reported that more than one million people are affected by echinococcosis globally each year and in 2015, 19300 deaths around the world. Treatment of patients infected with echinococcosis and the livestock industry damage is costing 3 billion dollars yearly [5]. Accurate molecular identification and *Echinococcus* genotyping is depending on tissue samples as an important source. Tissue samples can be obtained from cystectomy in spite of its invasive and can be used to confirm the cyst type through histopathology [6]. In endemic areas the diagnosis of hydatid cyst disease infection is carried out through the confirmation of a cyst mass in individuals who were exposed to sheep and dogs. Computed tomography (CT), Magnetic resonance (MRI), and ultrasonography (USG) are considered non-invasive imaging tools and used for detecting and defining the scope and condition of avascular cysts in most organs. The detection of hydatid cysts in the lungs can be done by radiography, but, calcification is necessary for visualization in other organ sites. Screening, clinical diagnosis, and monitoring of treatment of liver and intra-abdominal cysts were managed by ultrasonography.

Detecting specific serum antibodies is useful in the confirmation of Hydatid disease using immunodiagnostic tests. The tests include enzyme-linked immunosorbent assay (ELISA) using hydatid fluid or purified antigens like Antigen B – cystic echinococcosis, immunoblot analysis, immunoelectrophoresis [7].

MATERIALS AND METHODS

Settings

We tracked 15 records of patients attending European Gaza Hospital, AL-Shifa Hospital, Al-Ahli Arab Hospital and Nasser Hospital (Histopathology Department).

Samples collection

We collected 8 paraffin block samples from the histopathology departments in the four hospitals and two hydatid cysts directly obtained from the operation room (Figure 1) in the period from Jun, 2013 to August, 2019. Those samples were for patients who underwent surgery and were confirmed by histopathology. We collected information such as age, sex, infected organ and symptoms for each patient. Each paraffin section measures 8-10 µm was placed into 1.5 mL microtubes.

Samples examination

Histopathology

Thin tissue sections were de-paraffinized by pouring 1 mL of xylol on each sample, then incubate at 37 ^{o}C for 10 min, centrifuged at 1-500 g for 5 min. This stage was performed twice, and samples were rehydrated using 70%, 80%, 90%, and 100% alcohol, and for molecular diagnosis purposes [8].

Samples examination was carried out using three methods: histopathology, clinical presentation and molecular diagnosis. Hydatid cyst tissue from the liver was excised and kept in 10% formalin after surgery. Each tissue was dismantled into pieces of thickness 2–3 mm and washed underwater for a few hours, then it was dehydrated in descending degrees of ethanol and cleared in benzene, and embedded in paraffin. Tissue sections of 4–5 µm thickness were stained using the Harri's Hematoxylin and Eosin method [9].

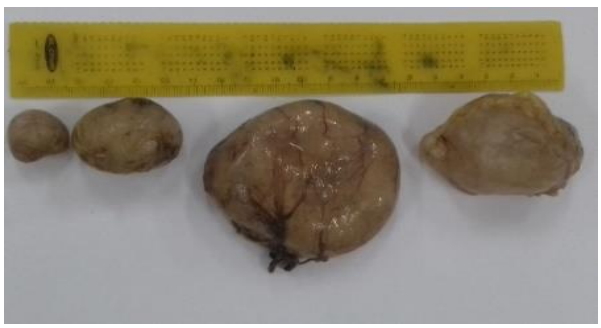

Fig-1: Gross examination Hydatid cysts from the woman in Gaza Hydatid, cyst wall consisting of an avascular, eosinophilic chitinous laminated membrane (Haematoxylin and eosin stain)

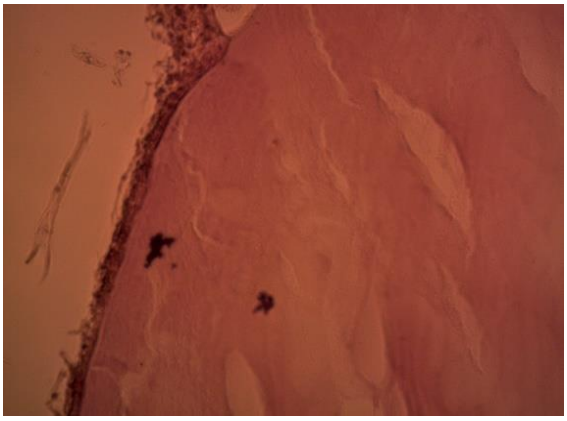

Fig-2: Gross examination Hydatid cysts from the woman in Gaza Hydatid, cyst wall consisting of an avascular, eosinophilic chitinous laminated membrane (Haematoxylin and eosin stain)

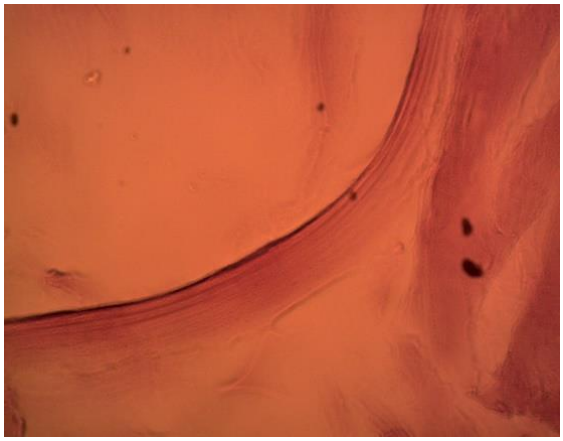

Fig-3: Gross examination Hydatid cysts from the woman in Gaza Hydatid, cyst wall consisting of an avascular, eosinophilic chitinous laminated membrane (Haematoxylin and eosin stain)

Molecular diagnosis

DNA extraction from hydatid cyst in liver tissue was done, where DNA was extracted using a DNeasy spin column kit for tissue (Qiagen, Hilden, Germany) by the manufacturer's instructions. Eluted DNA was stored at -20 ^{o}C until use.

Amplification of target DNA from cyst material *Echinococcus granulosus* PCR protocol (G1 PCR)

Amplification of target DNA from hydatid cyst material for PCR protocol (G1 PCR) was pursued. Amplification was performed in a 50 µL reaction volume with 5× manufacturers Flexi reaction buffer (Promega Ltd.), 200 µM of each deoxynucleoside triphosphate (dNTPs; Bioline), 0.3 µM of each primer (Eg1F81, 5′ GTT TTT GGC TGC CGC CAG AAC 3′ and Eg1R83, 5′ AAT TAA TGG AAA TAA TAA CAA ACT TAA TCA ACA AT 3′), 2 mM $MgCl_2$ and 2.5 GoTaq polymerase (Promega Ltd.). The reaction was started with an initial incubation of 5 min at 94°C for 1 cycle, followed by 36 cycles each consisting of 30 s at 94°C, 50 s at 62°C, and 30 s at 72°C to amplify a species-specific 226 bp fragment. This protocol was

according to [9] PCR products were resolved on a 1.5% agarose gel and viewed in a G-box imaging system (Syngene).

DNA sequence analysis

The sequences of nucleotides were analyzed using the FinchTV software package (Geospiza, Seattle, WA) and compared with those in the GenBank database through the use of BLAST software (www.ncbi.nlm.nih.gov/BLAST/).

RESULTS

The present study included 15 patients with hydatidosis with ages ranging from 7 to 69 years old. The isolated hydatid cysts' sizes ranged from 3.5 cm to 22.5 cm. The patients information with hydatidosis are presented in Table 1.

Table-1: Demographic characters of the patients with hydatidosis

Sex	**No.**	**%**
Male	8	53.3
Female	7	46.7
Residence		
Rafah	2	13.3
Khanyounis	4	26.7
Midzone	2	13.3
Gaza	7	46.7
Referral hospital		
Gaza Euorepean Hospital	6	40.0
Ahlia Arab Hospital	1	6.7
Al-Shifa Hospital	7	46.7
Nasser Hospital	1	6.7

Table 2 demonstrates the clinical /radiological findings indicating that the liver was the most affected organs among other organs (46.7%). In addition, 60% of patients complained of loss of weight.

Table-2: Clinical findings

Affected organ	**No.**	**%**
Liver	7	46.7
Spleen	1	6.7
Intra-abdominal	1	6.7
Lung	4	26.7
Uterus	1	6.7
Liver and kidney	1	6.7
Clinical symptoms		
Jaundice	2	13.3
Abdominal pain	2	13.3
Vomiting	1	6.7
Diarrhoea	1	6.7
Loss of weight	9	60.0
Discomfort	5	33.3
Loss of appetite for the past 4 months	1	6.7
Abdominal distension	1	6.7
Upper Abdominal fullness and mild tenderness	2	13.3
Mildly enlarged liver	2	13.3
Right liver lobe pain	1	6.7

Management procedure for the patients with hydatidosis is presented in Table 3, while the investigations are presented in Table 4.

Table-3: Management procedure for the patients with hydatidosis

Type of management	**No.**	**%**
Surgery	8	53.3
Albendazole	4	26.7
Excisional biopsy liver	1	6.7
Liver left lobe excision	1	6.7
Right lung Excision	4	26.7
Ciprofloxacin	1	6.7
Uterus excision	1	6.7

Table-4: Investigations are done for the patients with hydatidosis

Type of management	No.	%
CT for patient	3	20.0
Serological test ELISA	1	6.7
Plain X-ray	1	6.7

The amplified target 200bp DNA generated from *Echinococcus granulosus* from Human tissue (Fig 4).

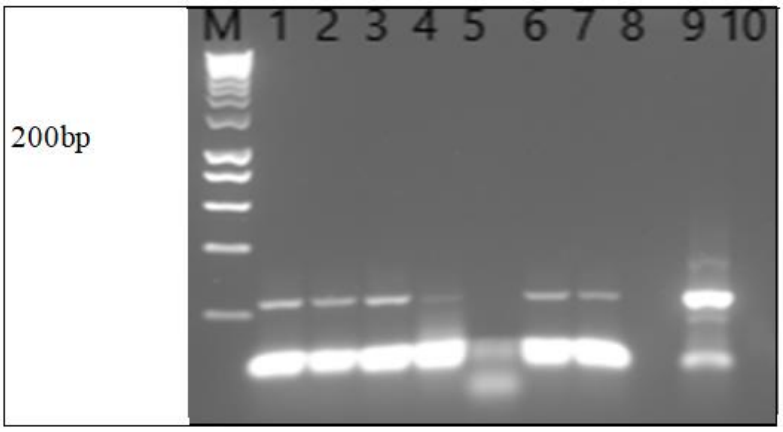

Fig-4: Gel electrophoresis of PCR product generated from *Echinococcus granulosus* from Human tissue Lane 1 (marker) 1, 2, 3, 4, 6,7 positive samples, 5, negative sample then 9 positive control

DISCUSSION

Hydatid cysts can affect any organ in the human body, where 50–70% of cases were in the liver as the most common, the lungs (20–30%), while the other organs are less discriminated [10].

The clinical cases of hydatidosis in the present study were diagnosed by the clinical presentation of the disease, histopathological identification, and molecular techniques. All cases were presenting clinical symptoms. The study showed that 46.7% of cases were primarily in the liver, followed by 26.7% presenting with lung cysts. Due to the lack of advanced diagnostic resources in such poor settings of the Gaza Strip, diagnosis of hydatidosis is determined by clinical presentation and histology.

The PCR amplification of the eight samples confirmed that these tissue samples were hydatid cysts. The sequencing results showed that one genotype of *E. granulosus* (G1) is accountable for human surgically-treated hydatid cyst in the Gaza Strip, with genotype G1 predominating.

The examination of hydatid cyst disease serologically using Antigen B5 is not available in the Gaza Strip settings, but hydatid cyst disease is normally confirmed in Gaza hospitals by the clinical picture, and histopathology where molecular diagnostics are very expensive in such a poor area.

The hydatid cyst disease is not so prevalent in the Gaza Strip but another two reports confirm the presence of this zoonotic disease. *E. granulosus* was observed from the adult stage in different countries as the first known molecular confirmation from Gaza and the Falkland Islands [9]. *Also it was the* most prevalent species causing human Cystic Echinococcosis in the Al-Khalil district in the West Bank and the Gaza Strip [11]. In Gaza Strip it was reported that 30 cattle and sheep were investigated for hydatid cysts, where 14/30 (46.6%) were positive for hydatidosis and 6/38 of examined dogs (15.7%) were positive for *Echinococcus granulosus* [12].

In the present study, eight cases of the tracked patients were subjected to surgery. Percutaneous treatment of the hydatid cyst with the PAIR (Puncture, Aspiration, Injection, Re-aspiration) technique is a treatment option for hydatid cyst disease according to WHO [13, 14].

LIMITATIONS: Purchasing diagnostic material from outside Gaza was a challenging matter.

CONCLUSION

Hydatid cyst disease occurrence is confirmed in the examined human tissue samples and belongs to genotype G1.

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