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Annals of Clinical Pathology

Research Article

Study of Hypertonic Saline Effect on the Hydatid Scolex under *In-vivo* Condition

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Submitted: 23 May 2016

Accepted: 10 July 2016

Published: 11 July 2016

ISSN: 2373-9282

Keywords

- Hypertonic saline
- Scolicidal agent
- Hydatid cyst

Abstract

Hydatid cyst is a parasitic disease caused by Echinococcus. New methods to treat hydatid

cyst have been suggested recently. Echinococcosis may infect multiple organs such as liver and lungs. Surgical treatment is usually used to treat large cysts that respond poorly to the medical management. Cyst content spillage is inevitable during cyst cavity drainage or cyst wall removal. Various measures are preferred by surgeons in order to prevent abdominal contamination.

24 patients entered this study according to our inclusion and exclusion criteria. During the surgery, 4 samples were taken: one sample taken from the right sub-diaphragmatic space after being washed with normal saline in the beginning, next sample drown directly from the cyst through a needle, third one taken from the cyst cavity after being exposed to impregnated gauzes of hypertonic saline, and the last one aspirated from the right sub-diaphragmatic space after being washed with normal saline before closing the incision.

Only one patient's first sample had a positive result (live scolex). 45% of the second samples were positive. None of the third samples showed a positive result. In the final analysis, 96% of the fourth samples were negative. This survey demonstrated that 20% hypertonic saline has high efficacy in destroying live scoleces in the cystic cavity.

INTRODUCTION

Hydatid cyst is caused by larval stage of *Echinococcus granulosus*. It is a worldwide zoonosis disease. This parasite is an important health issue and causes economical load in domestic animals especially in developing countries. Many patients infected by hydatid cysts are asymptomatic, even into advanced stages. Exposure to the parasite is specified through the amount of ingested foods or water contaminated by the feces of a proven vector [1]. *Echinococcus granulosus* is widespread through many regions of Asia including Middle East countries and some regions in Iran [2].

Echinococcus involves various organs such as liver (63%), lungs (25%), muscles, bones, kidneys, brain, and spleen (12%) [3]. Cysts larger than 5 cm are the most symptomatic due to their pressure effect. Some symptoms are abdominal pain, jaundice, biliary rupture, and urticaria. Considering the clinical symptoms, the diagnosis is followed by imaging techniques such as ultrasonography and CT scan. Essentially, it is confirmed by immunological studies such as ELISA [3].

Some studies showed that cysts failed to respond well to drug treatment, although Mebendazole and Albendazole might destroy simple cysts in 50-80% of cases. In case of drug treatment failure, the surgical management is the remaining option. The main purpose of the surgical treatment is minimum visceral sacrifice [4]. Some authors claim that the dissemination of protoscolex-rich fluid during surgery is a major cause of recurrence [5,6]. Scolicidal agents are injected into hydatid cysts in order to prevent relapse [7]. Intrahepatic relapse after surgery was seen in 10% of the patients [8]. Liver cyst recurrence is caused by microscopic leakage of live parasites, failure to remove all live cysts because of unavailable or difficult to reach locations,

and leaving a residual cyst wall at the initial operation. The latter was particularly true among patients with long-standing active cysts where there may have been infiltration through the original peri-cyst [9,10]. As mentioned, one of the causes of recurrent cyst is the residual protoscoleces [11]. Primary peritoneal hydatidosis is rare (2%) [13], and the mechanism of this infection is unknown. While a surgical approach is the definitive treatment, surgeons always face the danger of leakage or puncture of hydatid cyst during the operation. So, majority of surgeons recommend using impregnated gauzes with scolicidal agent around the cyst before it is discharged. Hypertonic saline is the most common scolicidal agent. Majority of studies, in the case of hypertonic saline, have been done in *in-vitro* field. There is a difference between *in-vivo* and *in-vitro* condition [13-16]. For instance, in *in-vivo* condition hydatid cyst fluid spillage is going to happen repeatedly. Also, surgical instruments such as suction, and surgical gloves are usually contaminated by the cyst fluid. Furthermore, laminated layer extraction is commonly associated with cyst fluid spillage [17]. Various studies investigated the efficacy of saline hypertonic in *in-vitro* field, despite the fact that no statistical analysis evaluates its efficacy in *in-vivo* field [15,18]. The purpose of this study is to evaluate the scolicidal efficacy of hypertonic saline during hydatid cyst surgery.

MATERIALS & METHODS

Patients with CE2 and CE3b cyst stage (according to WHO) participated in this study at the Alzahra Hospital, Isfahan University of Medical Sciences, Iran from February 2012 to December 2014.The diagnosis was based on the history, physical examination, ultrasonography, CT scan, and serological findings. Before the operation, all the patients received Albendazole (15 mg/kg/day divided into two doses, to maximum 400 mg orally) for 5 days. They continued the medical treatment for three months after the surgery.

Laparotomy was performed through a midline or subcostal incision. After opening the peritoneum, abdominal area was examined for the evidence of cyst rupture. Our exclusion criteria were multiple liver cysts, recurrent hydatid cyst, and cyst with biliary contamination or inadvertent cyst rupture during the operation. At first, the abdominal region was washed by normal saline and a 50 cc sample was drown from the fluid under the right sub-diaphragmatic region (Sample 1). Then the liver area containing the cyst was surrounded by impregnated gauzes with 20% hypertonic saline. A 16G needle was inserted into the cyst and 20 to 50 cc was removed as our second sample. Then, the remaining contents were evacuated by suction through the needle. After suctioning the whole cyst, it was unroofed and the germinal layer was removed completely. Also, a part of the germinal layer was sent to our pathology lab. Then, the impregnated gauzes with 20% hypertonic saline were placed in the cyst cavity for 15 to 20 minutes. After removing the gauzes, the cavity was washed with normal saline and a 50 cc sample was sent to the laboratory from the cyst cavity (sample 3). According to the cyst location and content, various methods such as omentoplasty, cyst wall partial resection, capitonage, and simple drainage were used to manage the cyst cavity. In the last step, the whole abdominal area was washed with normal saline and a 50 cc sample was taken from the right sub-diaphragmatic space as Sample 4. The abdominal wall was repaired finally.

All the samples were transferred to the laboratory in less than two hours in a plastic container in order to prevent exposure to heat or light. Data was collected and analyzed by SPSS software v22.

RESULT

61 patients with hydatid cyst were admitted in Alzahra hospital during our study. 33 patients having uncomplicated hydatid cysts were chosen based on their ultrasound findings to be CE2 or CE3b. 9 patients were removed from this group because of liver cyst recurrence (4 candidates), multiple liver cysts (3 candidates), cyst fluid biliary contamination (4 candidates), and cyst rupture (1 candidate). 24 patients met our inclusion and exclusion criteria. As it was mentioned previously, 4 samples were collected during the surgery and were sent to the laboratory. If a live protoscolex was seen in a sample, it was considered positive.

According to our analysis, 13 candidates were female and 11 were male with mean average age of 53and 41 respectively. 3 patients were diagnosed incidentally with liver hydatid cyst during abdominal ultrasonography due to other reasons. Their clinical symptoms were consisted of 1 acute abdominal pain, 19 wage upper abdominal discomforts, and 1 abdominal mass. Overall, 24 liver hydatid cysts were treated surgically. Our patients' CT-Scan findings about the cyst location and diameter are shown in Table (1). 10 cysts were located in one segment which 7^{th} segment was the most common. 14 cysts involved more than one liver segment. One of our cases was a 10-year-old girl with abdominal mass who had a liver cyst of 180*150 mm which involved all segments except segments II and III.

All the samples were centrifuged before macroscopic evaluation to find live scoleces. One of the patients' first samples was positive which accounted for 4%. This means that no infection was seen in 96% of the first samples while the second samples showed 45% of cases were infected. According to the third samples results, it was observed that none were positive. In the final analysis, 96% of the fourth samples were negative and one patient's sample showed a live protoscolex (Table 2). 22 Patients were followed regularly. Out of these patients, one was suspicious of recurrence based on the increasing size of the residual cyst cavity and serological findings, despite the fact that his samples were negative.

DISCUSSION

In areas where liver hydatid cyst is common, the recurrence of liver and intra-abdominal hydatid cysts is a major concern. Various methods are used to manage hydatid cysts besides surgery. Although the PAIR method is the choice treatment of CE1 hydatid cysts, a surgical intervention is usually used to manage CE2 and CE3b cysts. During most of the surgeries a macroscopic leakage or perforation was observed [16,17]. In the first stage of sampling, 96% of cases were negative which means one patient's first sample was positive (4%). It showed that our choosing and case collecting approach was suitable. No overt cyst perforation was detected in

Table 1: This table shows which liver segments were involved in patients by hydatid cyst.

Liver segment	Patients	Liver segment	Patients
VIII	1	II , III , IV	2
IV	2	V , VI , VII	1
V	2	II , III , IV , V	1
VI	1	V, VI, VII, VIII	1
VII	4	I , V, VI, VII, VIII	1
II,III	4	IV , V, VI , VII , VIII	1
V, VI	1	I , II ,III, IV, V, VIII	1
VI , VII	1	----------	----------

Table 2: This table shows how many samples were positive in each group.

Sample Number	Total samples	Positive samples	Positive percentage %
1	24	1	4
2	24	11	45
3	24	0	0

our primary surgical survey. So, micro-leakage was considered a possibility. There are reports of micro-leakage with or without clinical symptoms or signs [19-23].

It is stated that the leakage or spillage mostly happens during the primary aspiration and suction attempt. Most of the protoscoleces sink to the bottom of the cyst cavity due to gravity and cyst fluid might be sterile [35-36] which can be the reason why 55% of our second samples were negative.

Leaking sterile fluid into the abdominal area is one of the reasons that there are not much intra-abdominal hydatosis in majority of patients [24,25]. 45% of the second samples were positive which was expected because the samples were collected directly from the cysts.

Various methods have been used to prevent the spreading of protoscoleces in abdominal region during surgery [27]. The usual scolicidal agents are formalin [28],hydrogen peroxide[29], hypertonic saline[30], chlorhexidine[31], and absolute ethanol [32]. Ghafouri and his colleagues evacuated cysts by ultrasonography through the skin and filled it with hypertonic saline or ethyl alcohol. No cysts were detected in their two-year follow-up [11]. In one study, they examined Methyleneblue, hypertonic saline and normal saline effectiveness in *in-vitro* field. They concluded that Methyleneblue and normal saline had no effect on scoleces, but hypertonic saline destroyed 95-100% of them [10]. Scolicidal effect of 50% glucose solution, Cetrimide 0.5%, silver nitrate 0.5% and 20% hypertonic saline were examined in a survey. Highest scolicidal effect after 50% glucose solution was related to 20% hypertonic saline [9]. Numerous studies showed that *in-vitro* 20% hypertonic saline killed protoscloleces in a period of 15 to 20 minutes [18]. According to hypertonic saline, although there is much evidence of the scolicidal effect of hypertonic saline, majority of studies were not completed or most of them had been limited to *in-vitro* investigation [33-36]. Third sampling was conducted after cysts had been exposed to hypertonic saline and none of the samples were positive. Our method showed that hypertonic saline was effective in destroying the residual protoscoleces. No other studies investigated *in-vivo* effect of saline hypertonic in an open surgery.

In our study, one of the fourth samples was positive. As a matter of fact, the same patient with the first positive sample had a positive result in the fourth sample. This showed that the spillage happened before the operation although there was no macroscopic evidence. So, the analysis of the fourth samples results demonstrated that saline hypertonic destroyed the residual live scoleces in the right sub-diaphragmatic area. All in all, the negative results of the fourth sample and the follow-up indicated that although there is possible spillage and contamination during cyst aspiration or laminated layer removal, these protective measures destroyed live scoleces.

CONCLUSION

This survey demonstrated that 20% hypertonic saline had high efficacy in destroying live scoleces in the cystic cavity. Furthermore, protective measures taken by most surgeons before evacuation of the cystic cavity are effective against peritoneal dissemination of live scoleces in the sub-diaphragmatic area.

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Cite this article

Sanei B, Kolahdouzan M, Abedini L, Darani HY, Jafari HR (2016) Study of Hypertonic Saline Effect on the Hydatid Scolex under In-vivo Condition. Ann Clin Pathol 4(6): 1085.