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Research Article

The Efficacy of Ozonated Saline Solution against Protoscoleces of Cystic Echinococcosis in Liver Hydatid Surgery

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ABSTRACT
Echinococcus granulosus (Hydatid cyst) infection is one of the most common parasitic diseases that affect the liver. The cyst represent the larval stage of the parasite. The disease course is typically slow and the patients tend to remain asymptomatic for many years. The available and currently used treatment for *E. granulosus* infection include open surgery, percutaneous interventions, and pharmacotherapy. Ozonated saline solution is commonly used as a safe and efficient disinfectant for treatment of infections that occur during deep wounds and injuries. It is a potential adjuvant therapy to treat wounds and effective way of sterilization. The aim of the present study is to use ozonated saline solution *in vitro* for killing hydatid protoscoleces.
Materials & Methods: Samples of hydatid fluid were collected from hydatid cysts obtained from patients operated on due to liver hydatid cysts after operations for liver cystic echinococcosis. They were treated separately with the normal saline and ozonated saline. Then, the samples were examined microscopically for viability of protoscoleses by using 0.1% eosin stain.
Results: All protoscolices were dead after treatment with ozonated saline solution, i.e. 100% lethal effect, as they stained red after absorbing the eosin stain used in the viability test. Whereas non-azonated saline solution had no scolicidal effect. The ozonated saline solution showed a potential to kill the hydatid protoscolices *in vitro* which could be tried as a new method *in vivo* during operations of hydatid cystectomy without fear of toxic effects. Ozone is a safe non-toxic gas which becomes oxygen afterwards.
Conclusion: As per the author's best knowledge, this is probably the first research to prove the *in vitro* efficacy of of ozonated saline solution in the management of hydatid cysts.

Keywords: Ozonated saline, Protoscoleces, hydatid fluid, liver hydatid, surgery

INTRODUCTION

Hydatid cyst disease (cystic echinococcosis) is a common zoonotic disease of worldwide spread. It is caused by the larval stage of *Echinococcus granulosus*. The adult worms live in the intestinal lumen of dogs.While cattle, sheep, horses and humans harbor the intermediate larval stage which develops into hydatid cysts in the liver and lungs and other organs. Infection occurs after the ingestion of infective ova through contaminated food and water. Surgical procedures are the preferred therapeutic methods however the chances of intraperitoneal spillage of scolices should be considered and managed to prevent recurrence and dissimination of cysts, which is done using proper scolicidal agent by intracystic injection and to prevent reimplantation of scolices. Ozonated saline is used as a safe and efficient disinfectant for treatment of infections that occur during deep wounds and injuries. Ozone has been widely used in treatment of biofilms that develop during deep wounds. Ozone as a gas or dissolved in water works as an excellent disinfectant for mucosal or cutaneous infections. The oxidation potential of ozone is 2.07 volts which ranks it as one of the strongest oxidents for water therapy. Ozone has high oxidative activity that oxidizes and penetrates bacterial cell wall with consequent oxidation of all essential components within bacteria (DNA, RNA, proteins and enzymes) and eventually leads to cell lysis (1-4). This bactericidal mechanism of action differs from other agents like chlorine, which diffuse into the cells to cause several enzyme defects (3). The ozone kills bacteria about 3200 times faster

than the chlorine (5). Ozone is a bluish gas that has a boiling point at -112 C (1). It dissolves partially in water at atmospheric pressure and temperature and is 13 times more soluble in water than does oxygen. The main benefit of ozone is its clean character, because it only oxidizes materials, with forming almost no byproducts. Because ozone has a strong, recognizable odor, very low concentrations will soon be perceived. This makes it generally safe to work with ozone. In a test involving planktonic *Staphylococcus aureus* biofilm, it was observed that exposure to ozone saline was able to reduce 99% of bacteria or planktonic biofilm as observed within 15 min of exposure to ozonated saline. It was thus concluded that ozonated saline is a potential adjuvant therapy to treat wounds and effective way of sterilization too (7). Currently, many options have been proposed to evaluate alternative procedures to kill protoscoleces of hydatid cysts. New alternative strategies to control its infection are necessary since the available options may cause various adverse effects in liver function, headache, vomiting, nausea etc. induced by the medicines used. The available and currently used treatment for *E. granulosus* infection include open surgery, percutaneous interventions, and pharmacotherapy. The surgical treatment is usually done by cystectomy after intracystic injection of scolicidal agents to prevent intra-abdominal spillage and dissemination of hydatid duaghter cysts precursors (protoscoleces) during the operations. For this purpose numerous agents of various characters (formalin, hypertonic saline, ethyl alcohol, povidone iodine, albendazole, acids, garlic extract and many herbal extracts), were used however toxic outcomes and hepatobiliary complications accompany the use of most of these agents (8-10). With this background, the present study aims to evaluate the *in vitro* effectiveness of ozone on hydatid cyst protoscolices.

MATERIALS AND METHODS

Collection of hydatid fluid: In the present study, the hydatid cysts were obtained from patients operated on due to liver hydatid cyst disease. The hydatid fluid of these cysts was used to evaluate the effect of ozonated saline solution on viability of protoscolices. The samples of hydatid fluid were kept in sterile dark containers and transported to the laboratory to be used for further study.

Characterization of protoscolices : Hydatid fluid was aspirated and evaluated for the presence of viable and acceptable number of protoscolices by examining a sample of the fluid under direct microscope. Then eosin stain was used to detect and differentiate the viable from dead protoscolices,(see viability test below). The samples were diluted by adding physiological normal saline 4:1 ratio to maintain viability of protoscolices and stored in the refrigerator at 4 ◦C.

Preparation of ozonated saline: Ozonated saline is prepared by bubbling ozone-oxygen mixture into a bottle containing 500 ml of normal saline (0.9% sodium chloride) for 30min. The ozone gas is bubbled into the saline upto the saturation level and kept in refrigerator till use in a closed dark container (6).

Scolicidal activity of ozonated saline solution: About 1ml of hydatid fluid concentrate was added to 100ml of freshly prepared ozonated saline fluid and mixed gently by shaking. Control reaction contains 1ml of hydatid fluid concentrate in 100 ml of non-ozanated saline. Both of the mixtures were kept for incubation at 37 C for 20 min. After incubation, both groups of samples were examined for the viability of the parasites. The procedure was repeated three times.

Viability Test: The viability of the protoscolices of *Echinococcus granulosus* hydatid fluid samples was tested by using eosin aqueous solution 0.1% (1 gm eosin stain powder dissolved in 1000 ml. of distilled water). After mixing the sample of hydatid fluid with eosin solution, dead protoscolices absorb the stain and become red colored while live ones remain colorless.The protoscolices mortality rate is determined as percent of dead to total number (11).

RESULTS

In the present study, the effect of the ozonated saline solution was evaluated depending on the viability of the parasites within hydatid fluid. All protoscolices were dead after treatment with ozonated saline solution, i.e. 100% lethal effect. Hydatid cyst protoscolices were observed through microscope. Figure 1 shows viable hydatid cyst protoscolices after treatment with the eosin stain. In the normal saline, the protoscolices are alive while after treatment with the ozonated saline, they appear to be dead.Encysted protoscolix appeare viable, showing hooklets, in hydatid fluid (Figure 2A). Hydatid protoscolices as seen within the transparent laminated wall of the newly excised cyst (Figure 2B).

DISCUSSION

Many Health practicisioners have advised killing hydatid cyst protoscolices using hypertonic saline

solution (12,13). The dose of ozone used in ozonated saline solution is very low and calculated as per weight of the patient. As far as repetitive low dose of ozone is used, it is much less toxic. It triggers Nrf2 response. Damage to DNA is reversible and the cells quickly recover the genotoxic effect. The Nrf2 system contributes to various pathologies like inflammation, cancer, renal problems etc. (12). The administration of ozonated saline is probably safe and easy to perform since there is no risk involved. Surgical procedures for hydatid cystectomy wheather by laparoscopy or open surgery often include injecting scolicidal agents into the cyst before excision to avoid its dissemination. Most agents used produce unwanted side effects like cholangitis, hypernatremia or billiary disease after the use of ethanol, hypertonic saline and others respectively. Injecting scolicidal solutions like hypertonic saline into the hyatid cyst in operative procedures is often practiced to avoid parasite spread during surgical procedures. Hypertonic saline soaked in sponges is helpful because it works as a mechanical barrier to prevent infection (14). A 20% hypertonic saline solution is effective and commonly used to neutralize the cyst content in the infected liver (13). The treatment lasts about 10-20 minutes to kill all scolices. In the present study the authors have used ozonated saline (0.9%) solution to eradiate the protoscolices, which provided comparatively effective scolicidal activity similar to hypertonic saline. Franchi et al. studied effect of 10 mg/kg/day albendazole on 448 patients with uncomplicated hydatid cyst for 6 months. They reported that about 74% of the patients had deterioration in their cysts, and 25% of patients showed persistence at the end of 6 months (15). However, large dose (100 mg/Kg) is essential to obtain desirable scolicidal results,but with more adverse effects. Saremi reported that the percutaneous methods were effective against type I and type II cysts (16). The chlorine in the form of hypochlorite has been used as a disinfectant for more than 100 years. However, hypochlorites are toxic to most of the microbes (17). The ozone was used in drinking water due to its rapid decomposition and greater oxidation potential than other disinfectants (18). Our results are in agreement with these results. Ozonated saline is often given in the form of drip or in the form of multiple injections. They are infused into the body tissues, given intra-rectally via a bag. All these forms are practiced as ozone therapy to prevent surgical infections. This type of therapy poses strong antiseptic properties as most of the bacteria, fungi and even viruses do not grow or proliferate in the presence of ozone. Treatment of gastrointestinal or skin infections by ozone saline is very popular. Ozone saline has the capacity to spread throughout the body and enables various metabolic and physiologic effects(19).. Ozone as a gas or dissolved in water works as an excellent disinfectant for mucosal or cutaneous infections (20). As per the author's best knowledge, this is probably the first report on the use of ozonated saline to kill the protoscolices *in vitro*.

CONCLUSION

The results of the present study indicate the highly effective scolicidal activity of ozonated saline *in vitro* against protoscolices of hydatid cyst, revealing the potential use of ozonated saline solution as an promising new scolicidal agent for use in hydatid cyst surgery. However , further in vivo studies are needed to confirm these results.

CONFLICT OF INTEREST

The authors declare no confict of interests, associated with the present study.

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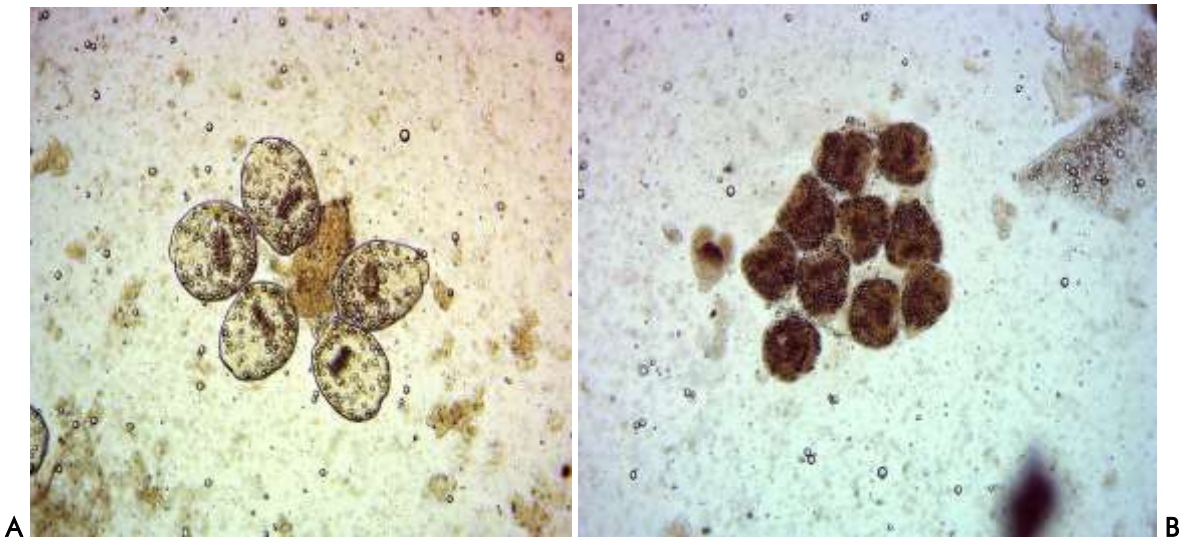

Fig.1: Viability test of eosin on a number of protoscolices that appear : A. viable, before and B. dead, after ozonated saline treatment.

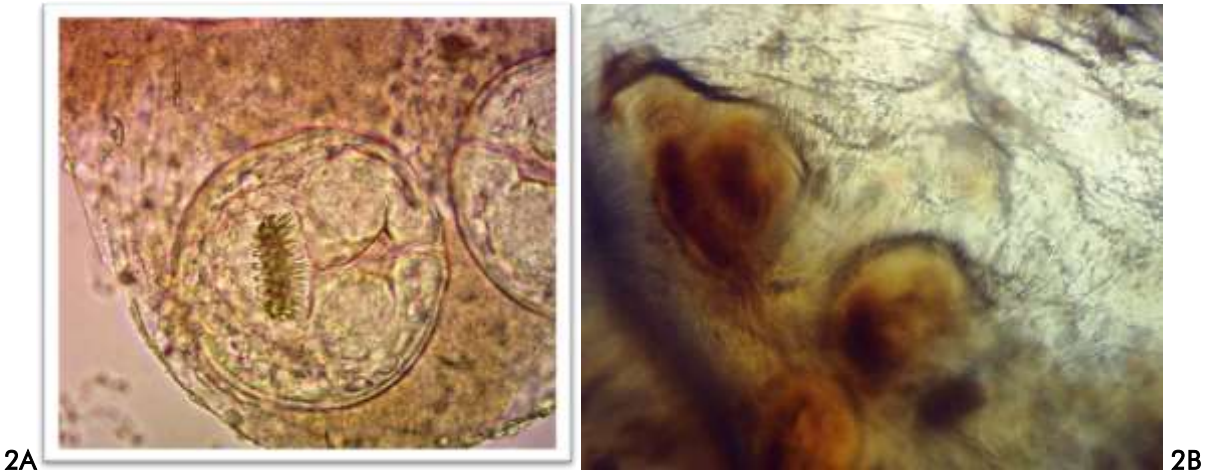

Fig.2: 2A:Encysted protoscolix appeare viable, showing hooklets, in hydatid fluid. 2B:Hydatid protoscolices as seen within the transparent laminated wall of the newly excised cyst .