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Original Article

Evaluation of the Protoscolicidal Effectiveness of Hypertonic Saline, Silver Nitrate, Ethanol, Using Sponge Pad Method and Injecting into Fertile Hydatid Cysts

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Abstract

Background: We aimed to evaluate currently protoscolices agents by using two methods including injection and impregnated sponges with protoscolices agents.

Methods: Hypertonic saline 20%, silver nitrate 0.5% and Ethanol 96% were injected into sheep hydatid cysts. Then, after different exposure times (5, 10, and 15 min), the protoscolices were aspirated from the cyst and viability of the protoscolices were evaluated microscopically using vital staining. In the second part, the live protoscolices were sprayed on the soaked sponge with the mentioned protoscolicidal agents. After different exposure times live protoscolices were counted microscopically. One-way ANOVA and Tukey tests were used for showing difference significant between studied groups.

Results: Cyst injection method showed that ethanol, Nacl and AgNo3 have 80%, 60% and 6% protoscolocidal efficacy, respectively ($P<0.05$). In sponge method the most and fastest protoscolocidal efficacy were observed in all used protoscolicidal agents.

Conclusion: Ethanol and NaCl and AgNo3 with sponge method is more effective than the injection of protoscolices agents in the cyst.

Introduction

Cystic echinococcosis (CE) is one of the most common zoonotic parasite disease in developing countries which is caused by larval stage of a cestoda worm called *Echinococcus granulosus* (1-3). CE is almost universally distributed, especially in Central and South America, Sub-Saharan Africa, Russia, China and Mediterranean countries. Factors such as migration have made the disease a re-emerging problem in Europe. Mortality rate is estimated at 0.2 per 100,000 population, with a lethality rate of 2.2-5% (4). The WHO has declared echinococcosis as a neglected disease (5, 6).

Four treatment strategy have been considered included surgery, PAIR (Puncture, Aspiration, Injection, and Respiration), drug therapy and observation (Wait and Watch strategy) (7, 8). The first treatment choice is surgery (9, 10) but, despite the advantage of surgical techniques, there is a risk of leakage and formation of secondary infection (9, 11). Recurrence of the disease has been reported in 2-25% of patients (11) during surgery. Therefore, it is important to present appropriate scolocidal agents and define effective exposure time (12, 13).

Different scolocidal agents have been used with variable efficacy and in some cases based on surgeon's experience (12). The use of impregnated sponge pads with scolocidal substances around the surgical site has been performed from past to present. Sponges act as a mechanical and chemical barrier (14, 15). However, no scolocidal substance completely kills the protoscolices inside the cysts (2). Hence, finding a more effective scolocidal substance with a shorter effect time is essential for this purpose (13). Currently, WHO-IWGE (Informal Working Group on Echinococcosis) recommends hypertonic saline 20% as the preferred protoscolicidal agents in surgery and hypertonic saline 20% or ethylic alcohol 95% in PAIR, but high-quality comparative studies concern to protoscolicidal agents are still awaited (2, 4).

In some studies the effect of scolocidal substances on live protoscolices were investigated (3, 10, 14, 16, 17). Besim and Khan examined the effect of scolocidal substances on the sponge pad after 15 minutes. Protoscolices sprayed on different soaked sponges with betadine, saline 20%, hydrogen peroxide 3%, ethyl alcohol 95%, and Chlorhexidine 10%. The protoscolices were inactivated after 15 minutes but the concentration of saline less than 20% was not effective(14, 16) . Yatim et al. indicated the volume of the hydatid was reduced by ethanol 98% and albendazole in injection method when compare with control group (18).

It is important to know which method has the best scolocidal effect for controlling secondary hydatidosis after surgery. Therefore the current study was conducted to investigate common scolocidal agents using sponge method and injection with considering the best exposure time with different concentrations and exposure times.

Materials and Methods

Current research was approved by Ethical Committee of Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1399.119 dated 02.05.2020).

Cysts collection

Hydatid cyst were collected from infected sheep in Ahvaz slaughterhouse. Before using of the cysts, it should be noticed that the cyst does not contaminate with bacteria and having live protoscolices.

Injection method

Thirty percent of the cyst fluid volume was aspirated with a syringe. The first approxi-

mately volume of the cyst was calculated following formula: ($V = 4/3\ \pi r^3$). Spherical cysts were preferably used. The same volume of aspirated cyst fluid was replaced with hypertonic saline 20%, Ethanol 96%, silver nitrate 0.5% and PBS as negative control. After 5, 10 and 15 minutes, the cyst fluid was aspirated and after staining protoscolices with eosin 0.1%, the live protoscolices were counted.

Sponge pad method

Four petri dish containing hypertonic saline 20%, ethanol 96%, Ag No3 0.5% and PBS were prepared and a piece of sponge pad were soaked in its. Then, 2000 protoscolices in 0.5 ml cyst fluid was added to sponge and left for 5, 10, 15 and 20 min. After the exposure times the sponges were washed with PBS solution and the number of live protoscolices were count after vital staining (Fig.1).

Fig.1: Sponge method. Soaking in substances and washing sponges in PBS.

Statistical tests

Kolmogrov-smirnov test was used for testing normal distribution of statistical data. ANOVA and Tukey's test were applied for showing significant difference between groups. Significant difference level was considered at $P<0.05$.

Results

Injection method

Ethanol, NaCl and AgNo3 had 80%, 60% and 9% scolocidal activity after 20 min respectively. The fastest killing effect was observed for Ethanol, NaCl and AgNo3, respectively (Fig. 2).

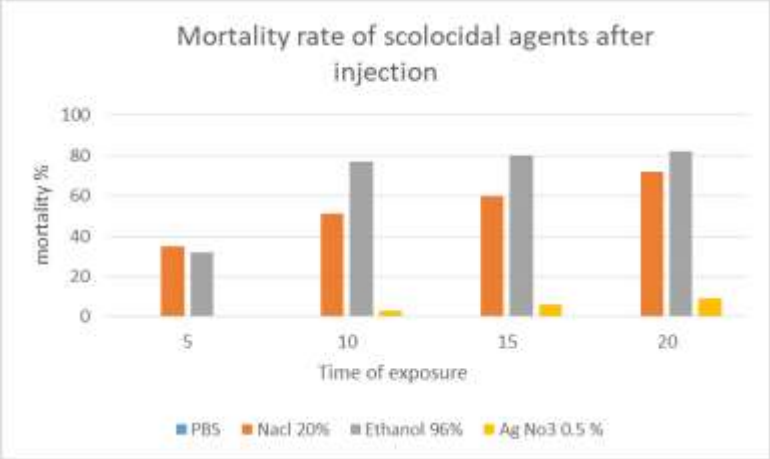

Fig. 2: Mortality rate of scolocidal agents after injection ($P=0.001$)

In sponge method the most and fastest scolocidal efficacy were observed for: NaCl, AgNo3 and ethanol respectively (Fig. 3). The comparison of scolocidal agents in injection and soaked sponge situation revealed that soaked sponge had more effective and fast scolocidal effects (Figs 4-6). The scolocidal activity for ethanol and NaCl were started at 5 min after exposure and increased with passing time but the killing effect of silver nitrate were began at 10 min after exposure and rises at 20 min later. Kalmogrov-Smirnov test showed a normal distribution in data and therefore for the next statistical analysis parametric test including ANOVA and Tukey test were used. ANOVA analysis indicated that there are significant difference within studied group (P<0.05). Tukey's test showed this difference are related to ethanol group and silver nitrate group (P<0.05). Tukey's test also showed significant difference between NaCl and silver nitrate groups (P<0.05). There were no significant differences between ethanol and NaCl groups (Table 1).

Table 1: Statistic Tukey's analysis in ethanol, NaCl and AgNo3 groups in scolocidal activity (injection method)

Group (I)	*Group (J)*	*Mean difference (I-J)*	*Std.Eror*	*Sig.*	*Lower Bound*	*Upper Bound*
Ethanol	NaCl	11.75000	11.23178	.568	-19.6092	43.1092
	AgNo3	65.00000*	11.23178	.001	33.6408	96.3592
NaCl	Ethanol	-11.75000	11.23178	.568	-43.1092	19.692
	AgNo3	53.25000*	11.23178	.003	21.8908	84.6092
AgNo3	Ethanol	-65.00000*	11.23178	.001	-96.3592	-33.6408
	NaCl	-53.25000*	11.23178	.003	-84.6092	-21.8908

*The mean difference is significant at the 0.05 level

Sponge pad method

Analysis of scolocidal activity in sponge pad method presented that all of the agents indicated fast killing effect on protoscolices and statistical tests showed there was no significant difference among studied groups (Fig. 3). The killing effect of all group were started at 5 min after exposure and continued up to 20 min

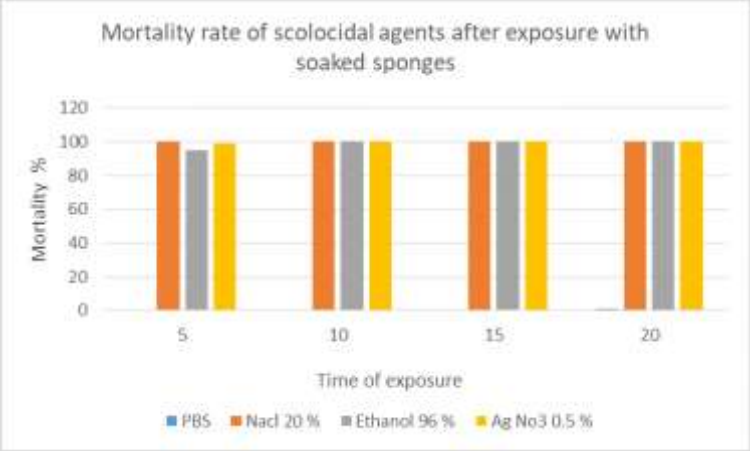

Fig. 3: Mortality rate of scolocidal agents after exposure with soaked sponges (**P**=0.405)

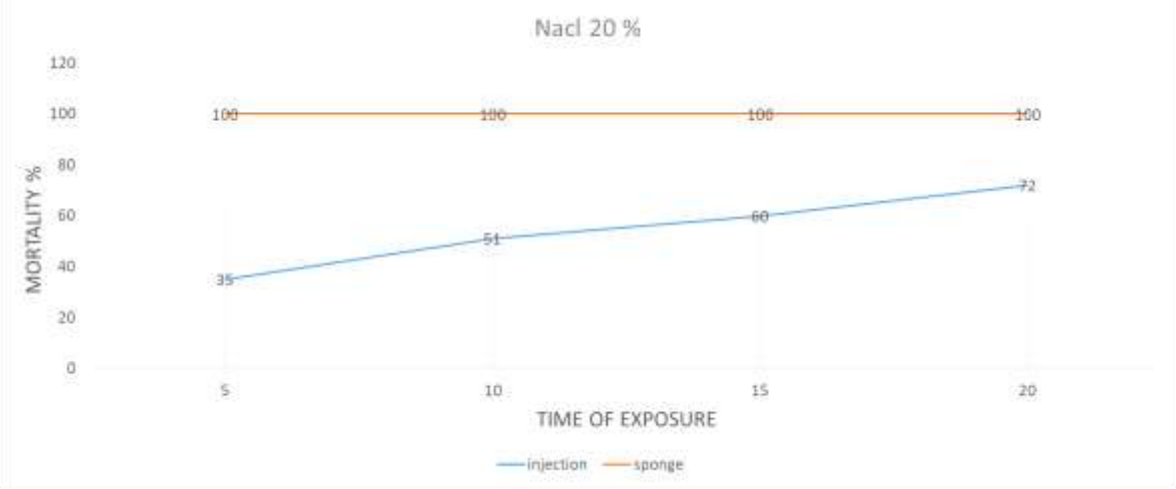

Fig.4: Mortality rate in 20% NaCl between injection and sponge methods (*P*=0.002).

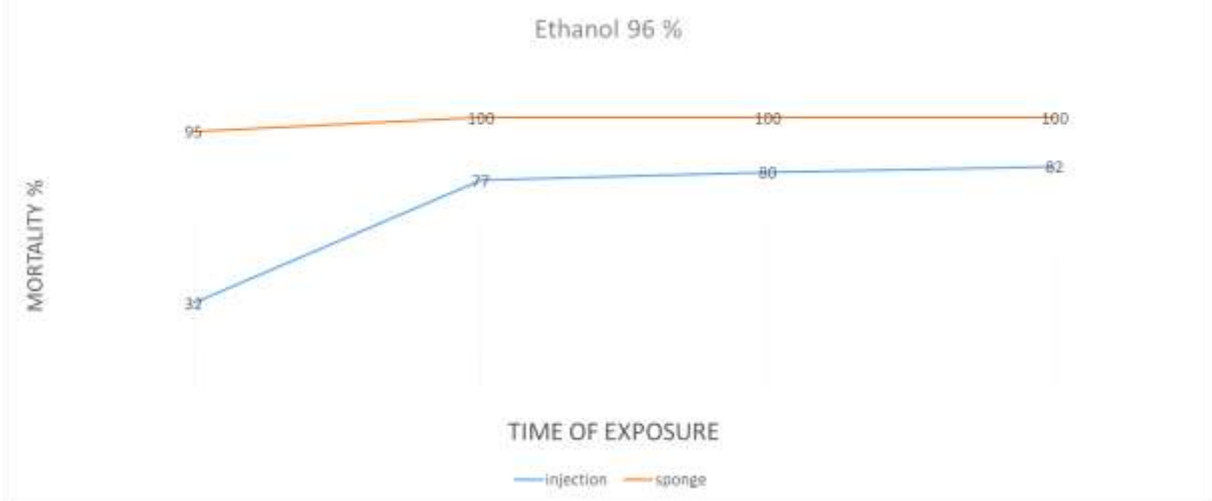

Fig.5: Mortality rate in 96% ethanol between injection and sponge methods (*P*=0.037)

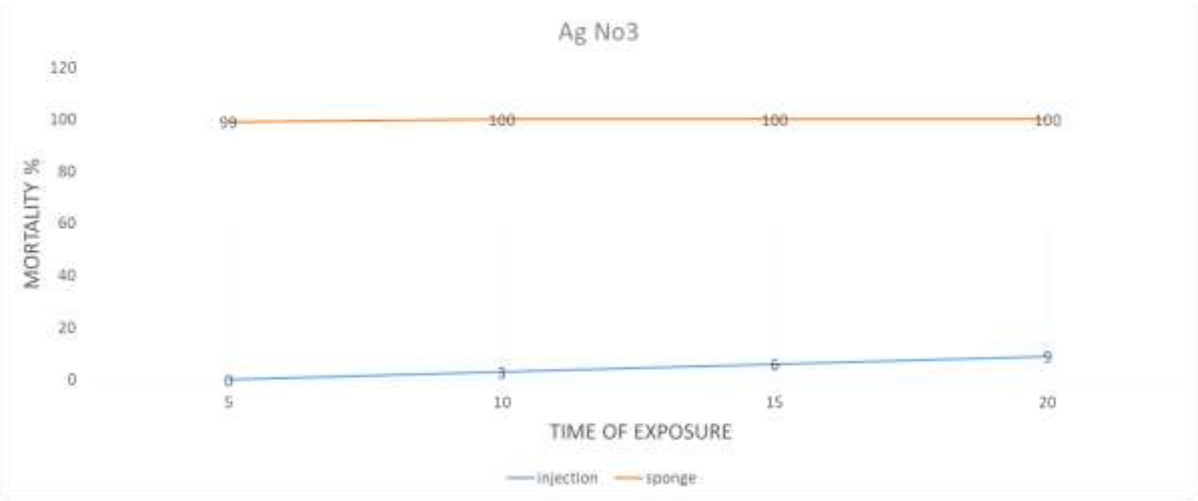

Fig. 6: Mortality rate in 0.5% AgNo3 between injection and sponge methods (*P*=0.000)

Discussion

Surgery is the first approach for CE treatment but there are some limitations including bacterial contamination after surgery, inaccessible cyst such as the formation of the cyst in myocardia and deep of brain, elderly and pregnancy situation. However one of the main problems in the surgery method is the recurrence and secondary cyst formation due to cyst spillage during surgery. As overall, recurrence rate have been reported 2-25% after hydatid surgery (9, 11).

Cyst drainage, germination layer destruction, prevention of cystic fluid leakage, disinfection and closing the cystic cavity are the main strategy in hepatic hydatid surgery (18).

In CE disease, the risk of spreading the cyst contents can be prevented by injecting a strong scolocidal agent such as Chlorhexidine. Although surgery is the first choice for liver hydatidosis, the perfect method for managing the remaining cavity and using scolocidal agents is still debated (15). Currently, injection of scolocidal agents into hydatid cysts is a common practice and many surgeons have abandoned this method due to a lack of objective evidence about the effectiveness and side effects of these substances (14).

The waiting time for the deactivation of protoscolices is important. Shorter time exposure of protoscolocidal agents would help surgeons to minimize time of surgery as much as possible. In most studies, NaCl 20% revealed to be an effective scolocidal material in a short time of exposure. Ethanol 96% and Silver nitrate 0.5% were also effective substances (3, 10, 14, 16, 17). These three substances are widely used in hydatid surgeries.

One effective way to prevent the spillage of protoscolices is to surround the surgical location with impregnated sponges with scolocidal material (15).

In the current study, after 5 minutes the mortality of protoscolices in injection method, was almost half of the effectiveness of the sponge method. Therefore, it seems that clinician should take sponge method as priority of treatment in comparison with injection method. In the other hand it is strongly suggested that injection method has always risk of rupture of the cyst.

There are no studies which compared these two methods at the same time. The most lethal and fast effects on killing protoscolices were observed for: NaCl, AgNo3 and ethanol, respectively in the sponge method. Furthermore, these results in injection method were Ethanol, NaCl and AgNo3, respectively. NaCl kills protoscolices faster than the other two substances. Therefore, the use of NaCl is recommended in surgeries where speed operation is important. Effectiveness was directly related to time in injection method but not for sponge method. With increasing time, the effectiveness of scolocidal agents in all three substances increased. The mortality rate after 10 minutes exposure of all materials was increased significantly (approximately double) and then has been increased over time slowly.

Comparison of the injection and sponge method indicated that the lethal effect of intra-cystic injection was significantly less than the sponge method ($P<0.05$). The reason for this difference could be due to diluting of the scolocidal agents in the cyst fluid and probably the effect of the germinal layer, interaction with substances within the cyst such as ionic substances, unknown inhibitors, and pH changes. The use of scolocidal agents in surgery is based on studies that have often examined the direct exposure of protoscolices in in vitro. In these studies, the protoscolices are contacted to pure scolocidal agents, and increasing the contact surface is likely to increase the effectiveness of the material.

There is controversy among surgeons opinion to which substance is more effective. Therefore, further studies with similar clinical conditions are necessary. Previous studies have been performed on various scolocidal agents and concentration (13, 17, 19), but in the present study, scolocidal agents have been

performed as routinely concentration used in practice.

In the current study, not only the rate of death and exposure time, but also, the type of active substance differs in two stages (sponge and injection stage). The results of Khan Study (14) was in disagreement with the current study. Although, there is no clear explanation in this regard but, it may be due to different protoscolices source in the two studies (human hydatidosis and sheep hydatidosis source).

The characters of favorite scolocidal agent is defined as: being potent in low concentrations , acting in a short period of time, chemical stabling in cyst fluid, not to be ineffective by diluting in cyst fluid, killing the protoscolices within cysts, without non-toxic effect, having low viscosity, to be available and easily and proper prices (3, 13, 20).

Conclusion

Ethanol and NaCl seems to be more reliable scolocidal substances in term of clinical application and the sponge method is more effective than the injection of scolocidal agents to cysts.

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Competing interests

None of the authors have any conflicts of interest to disclose.

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