The Toxic Effect of Magnetic Field on Protoscoleces of Hydatid Cyst in Vitro

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ABSTRACT

Background: Hydatidosis it is a zoonotic disease which is caused by the larval stages of different species of the tapeworms (Cestoda) of genus Echinococcus. Currently, the surgery is most definitive method of treatment for Hydatid Cyst, but always there is the risk of leakage or rupture during the surgery, therefore it is considered unreliable method.

Methods: Protoscoleces of hydatid cysts were placed at the center of 1.5 Tesla magnetic fields in 3 different intervals of 15, 30, and 60 minutes. In each stage, they were exposed to the magnetic field four times and the viability rate of protoscoleces was measured after each exposure period.

Results: The results showed a significant difference between viability rates for protoscoleces in case and control groups (P=0.004).In the first stage, when protoscoleces were exposed to the 1.5 Tesla magnetic field for 15 minutes between 1 and 4 times, it did not reveal any significant differences between case and control groups (P=0.793). In the second and third stages, protoscoleces were exposed to the magnetic field for 30 to 60 minutes respectively, it showed significant differences between case and control groups (P<0.05).

Conclusion: The findings of this study showed a decrease in the viability rate of protoscoleces exposed to the 1.5 Tesla magnetic fields for 30 and 60 minutes.

Keywords: Hydatid Cyst, Magnetic Field, Protoscolex.

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INTRODUCTION

Hydatidosis is a zoonotic disease which is caused by the larval stages of different species of the tapeworms (Cestoda) of genus Echinococcus. The mature worms of this parasite reside in the small bowel of carnivorous animals; such as dogs, wolves, foxes, and coyotes, where they disperse their eggs in the environment through their feces [1, 2]. The entrance of these worms to the body of herbivores, such as camels, sheep, goats, and cows, leads to the development of cysts in different organs, particularly kidneys and lung.

Human incidentally enters the life cycle of these worms as the intermediate host (biological dead end). Consequently, cysts develop in organs such as the liver, lungs, brain, kidneys, and bones [1, 3]. Prevalence of hydatid cysts depends on social habits, nutritional status and rates of human contact with canines. The most prevalence occurs in the third and fourth decades of life which is considered as his/her active age period [4, 5]. This disease imposes great financial burdens on the economy of world governments. In some countries the health care cost for each patient is estimated to be more than $2000 [6, 7].

Surgery is currently the most definitive treatment, but is always accompanied by the risk of leakage or rupture of the cyst during surgery. Moreover, if anti protoscolex agents are used during surgery, the fluid inside the cyst must be drained away first; which brings the risk of leakage to the neighboring tissues and recurrence of the disease; accompanied by development of such complications as

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cholangitis and inspissation of bile. Hence it is not considered as reliable method [1, 8].

Using drugs for eradication of the germinal epithelium of hydatid cysts and protoscoleces does not mean that the surgeon does not need prevention of leakage and spread of hydatidic fluid [3]. Furthermore, some patients are not suitable candidates for surgery due to the presence of different cystic complications in various organs, difficulty of access to the site of the cyst, or specific physical conditions [9].

Noticing the pathophysiologic significance of hydatid cysts, researchers have tried to find a suitable and practical way for controlling and treating hydatid cysts in patients. In this regard, non-invasive methods for example using electricity, laser, and radioactive drugs are more notable [1, 10-14].The aim of the present study was to investigate the lethal effect of magnetic field on the protoscoleces of hydatid cyst.

MATERIALS AND METHODS

SAMPLING

hydatide cysts were collected from kidneys and lungs of the infected animals slaughtered in Arak Slaughterhouse, after receiving permission from General Department of Veterinary of Markazi provienc, Iran . They were transferred to the Laboratory of parasitology. The protoscoleces of hydatid cyst were extracted in sterile conditions and were transferred to test tubes along with 6CC of the fluid of hydatid cyst.

SAMPLE PREPARATION

This experiment was carried out in 3 stages. In each stage, the tubes containing protoscoleces were placed in the 1.5 Tesla magnetic fields for 15, 30, and 60-minutes periods in four round. In each round, 20 test tubes were used as control and another 20 were used as case tubes.

THE EFFECT OF THE MAGNETIC FIELD

The magnetic field was generated by MRI device with 1.5 Tesla power. In the first round of the experiment, the following measures were undertaken:

- Placing five test tubes containing protoscolex in the magnetic field for 15 minutes and determining their viability rate.
- Placing five test tubes containing protoscolex in the magnetic field for 15-minutes in 2 rounds with 6-hour interval and determining their viability rate.
- Placing five test tubes containing protoscolex in the magnetic field for 15-minute in 3rounds with a 6-hour interval and determining their viability rate.
- Placing five test tubes containing protoscolex in the magnetic field for 15-minute in 4rounds with a 6-hour time interval and determining their viability rate.

In each step, counting was performed for each one of five test tubes in the control group. The second and third rounds of experiment were conducted similar to the first one; except its duration which were 30 and 60 minutes respectively.

Staining and morphological analysis

Eosin staining (0.1%) was used to measure viability rate of protoscoleces.

RESULTS

The comparison of the results between the case and control groups through paired t-test showed a difference in the viability rate of protoscoleces in two groups (P-value=0.004). In the first round, the mean viability rates of protoscoleces exposed to 1.5 Tesla magnetic field one to four times for 15 minutes were 67.3, 58.9, 67.9, and 66.2% respectively, whereas the mean rates of viability of protoscoleces in the control group were 68.4, 60, 68.8, and 67% respectively (table 1), which did not show a significant difference between the case and control groups (P-value =0.793). It should be noted here that in each stage, the viability rates for protoscoleces in the case group were compared to the values in the control group in the same stage.

In the second round, the mean rates of protoscoleces viability in the test tubes exposed to the 1.5 Tesla magnetic field for 30 minutes between 1-4 times were 96.5, 97.2, 95.3, and 98.8% respectively, whereas the viability rates for the control tubes were 97.5, 98.8, 97.9, and 100% respectively (Table 2).

It shows a significant difference between case and control groups (P-value =0.001).

In the third stage, the mean rates of protoscoleces viability in the test tubes exposed to the 1.5 Tesla magnetic field between 1-4 times for 60 minutes were 84.5, 83.7, 80.5, and 83.8% respectively, while the mean viability rates of protoscoleces in the control group were 87.5, 88, 86.5, and 85.5% respectively (Table 3). It indicates a significant difference (P-value =0.000). The difference was more notable than the second stage.

There was not a significant difference between the group that had 60-minute exposure for one time and the group that had 30-minute exposure for two times (P=0.432). In addition, there was not a significant difference between the group that experienced 60 minute exposure for 2 times and the group with 30 minute exposure for 4 times (P=0.193). It is demonstrated in table 2and 3.

Table1. The viability of protoscoleces in magnetic field for 15-minutes for 4 rounds.

No.test tube	round 1st 15 min		round2nd 15 min		round3 rd 15 min		round4 th 15 min	
	Case Group	Control Group	Case Group	Control Group	Case Group	Control Group	Case Group	Control Group
First tube	75.4	75.4	61.6	62.1	74.7	75.1	69.2	69.5
Second tube	68.6	69.1	50	52	52.6	52.8	48.6	49.2
Third tube	72	72	63.8	64	73.4	74	77.9	79
Fourth tube	78.2	80.3	73.2	74.2	69.3	72	66.5	67.2
Fifth tube	42.5	45	46	48	69.7	70.2	69.2	70.5
Viability mean	67.3	68.4	58.9	60	67.9	68.8	66.2	67

(P value = 0.793)

Table 2. The viability of protoscoleces in magnetic field for 30-minutes for 4 rounds.

No. test tube	round 1st 30 min		round2nd 30 min		round3rd 30 min		round4th 30 min	
	Case Group	Control Group	Case Group	Control Group	Case Group	Control Group	Case Group	Control Group
First tube	94.1	96.4	100	100	89.8	96.2	98.1	100
Second tube	98.3	99	89.3	95.3	94.5	97	97.2	100
Third tube	95.2	97	100	100	98.6	100	100	100
Fourth tube	95.3	95.5	99.1	100	95.8	97.7	98.8	100
Fifth tube	100	100	97.6	98.8	98	99	100	100
Viability mean	96.5	97.5	97.2	98.8	95.3	97.9	98.8	100

(P Value = 0.001)

Table 3. The viability of protoscoleces in magnetic field for 60-minutes for 4 rounds.

No. test tube	round 1st 60 min		round2nd 60 min		round3rd 60 min		round4th 60 min	
	Case Group	Control Group	Case Group	Control Group	Case Group	Control Group	Case Group	Control Group
First tube	87.6	92.3	81.3	84	69.9	76.5	90	93.2
Second tube	84.2	86	84.8	90.7	83.3	92.8	71.5	74.1
Third tube	79.8	83.8	82.5	87.6	83.3	87.6	86.1	87
Fourth tube	85.3	87	81.4	83.8	83.4	88.4	86	86
Fifth tube	86	88.5	88.8	94.1	83	87.3	85.4	87.5
Viability mean	84.5	87.5	83.7	88	80.5	86.5	83.8	85.5

(P value = 0.001)

DISCUSSION

The findings of current study showed that 1.5 Tesla magnetic field did not have any significant effect on the protoscoleces of hydatid cyst, if it is exposed in 15-minute even for 4 times, whereas the viability rate of protoscoleces reduces ; if they were exposed to the magnetic field in 30 and 60-minute for 4 times. Therefore, the duration of exposure to the magnetic field is a major determinant in mortality of protoscoleces which can be due to alteration and production of toxic materials in the cyst.

Furthermore, it seems that duration of exposure to the magnetic field is important. 60-minute exposure to the magnetic field is similar to30-minute exposure for two times; or 60 minute exposure for two times is comparable to 30 minute exposure for 4 times. Hence continuation and repetition of exposure duration did not bring about any significant changes in the effect of the magnetic field.

In the test tubes containing the case protoscoleces; which were counted again with a 6-16 hour intervals following transfer to the magnetic field, no significant changes were observed.

However, it is likely that if the viability rate of protoscoleces had been checked with a 3-5 day time intervals, more significant effects could have been observed.

In their investigation of the effects of magnetic field on red blood cells contaminated with malaria parasite, Holick et al found out that magnetic fields can both inhibit iron conversion pathway to hemozoin polymer and total trophocyte or schizonts plasmodium which produce food vacuoles containing hemozoin. [15]. In both cases, fluctuation in the intensity of the magnetic field leads to fluctuation in the amount of toxic hemozoin and as a result, damage to the organs of the parasite and osmotic disturbance, and errors in molecular arrangement lead to the parasite's death [16].

In another study, concomitant effects of magnetic field and polymyxin on the growth of Pseudomonas aeruginosa and Bacillus cereus were investigated. It was observed that 21 gauss magnetic field only changes the pattern of Bacillus cereus resistance to polymyxin [17].

In another study, the effect of magnetic field on the reduction of blood glucose has been investigated in rats. Although the rats blood glucose level did not change during exposure to the magnetic field; their blood glucose values were lower in the rats exposed to the magnetic field 10 days after exposure; compared to rats that had not been exposed to the magnetic field [18].

Up to now, there was no proven and definite hypothesis about direct impact or toxic effect of magnetic field on biologic materials [19].

Magnetic field does not have similar effect on all living organisms. In Kimbll's study, it was shown that the growth of yeasts decreases in the magnetic field [20], whereas Genkov et al have indicated that the growth of Trichomonasvaginalis rapidly increases in the magnetic field [21]. The findings of another study conducted by Triampoet al have also demonstrated that the morphology of leptospira undergoes changes in the magnetic field [22]. The difference in these findings can be attributed to the difference in the cells or living tissues used in the studies [23, 24]. Findings of another study showed that the growth of Bacillus subtilis increases in 150 gauss magnetic field, whereas it decreases in 300 gauss magnetic field [23]. Likewise, the growth rate of vibrio cholera increases in magnetic fields with intensities less than 400 gauss, whereas it decreases in 580 gauss magnetic field [24]. Several other studies have also referred to the fact that the intensity of the magnetic field and the duration of induction are considerable factors in viability rate or morphologic and genetic changes in living organisms [13, 25, 26]. Also, some researchers believe that the changes brought about by magnetic field are related to heat and sound vibrations of the electric magnets [1, 27], but in the present study, cooling systems were utilized in order to prevent the heat generated by the magnetic field and increased temperature in the living organisms.

It is possible that the solution that protoscoleces are placed in and exposure to the magnetic field produces other more toxic effects of the magnetic field on protoscoleces

of hydatid cyst through changes in the exposure periods. Therefore, further studies with changes in methodology, the intensity of the magnetic field, or duration of exposure are suggested.

CONCLUSION

The findings of present study showed decrease in the viability rate of protoscoleces exposed to the 1.5 Tesla magnetic fields for 30 and 60 minutes.

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