Türkiye Parazitoloji Dergisi, 34 (1): 21 - 23, 2010
© Türkiye Parazitoloji Derneği

Türkiye Parazitol Derg.
© Turkish Society for Parasitology

Production of Experimental Hydatid Cyst in the Eye, Peritoneum and Liver of BALB/C Mice

Javad MOUSAVI[1], Khosrow Hazrati TAPPEH[2]

[1]Department of Surgery, Imam Hospital, Urmia University of Medical Sciences, Urmia;
[2]Department of Parasitology & Mycology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran

SUMMARY: As the main treatment for this infection is surgery, the surgery team personnel are at the risk of the protoscoleces released from the hydatid cysts (HC) of patients. The first goal of this study was to determine the probability of the production of ocular HC in mice due to the fluid of the aspirated protoscoleces from the sheep liver with HC. The second goal of this study was to produce HC in the peritoneum and liver, in order to gather more information for future studies on hepatic and peritoneal HC treatment procedures. For the first goal of this study, different concentrations of protoscoleces were prepared and injected into the eyes of 60 mice. After 20 weeks, 10 of the 60 mice of this group died. The remaining 50 mice were examined by a surgeon under the anesthesia. There weren't any symptoms of HC in the eyes and around it. For the second goal, 39 new mice were separated into three sub groups and 0.5 ml of protoscolex solution was injected intraperitoneally. After 20 weeks, they were anesthetized and their peritoneum, intestines and liver were examined. HC was seen in the peritoneum and liver of 6 mice.

Key Words: Eye, Liver, Peritoneum, Experimental hydatid cyst.

BALB/C Türü Farelerin Göz, Periton ve Karaciğerlerinde Hidatik Kist Oluşturulması

ÖZET: Hidatik kistin esas tedavisinin cerrahi olması nedeniyle cerrahi personeli her zaman için hastalardan protoskolex ile bulaş riski altındadır. Bu çalışmanın amacı, hidatid kistli koyun karaciğerinden aspire edilmiş protoskolex içeren sıvının farede oküler hidatid kist oluşturma olasılığını saptamaktır. Ayrıca, tedavi uygulamaları için ileride çalışmalar yapabilmek amacıyla periton ve karaciğerde hidatid kist üretme konusunda daha fazla bilgi edinilmesi de düşünülmüştür. İlk amaç için, farklı konsantrasyonlarda skoleksler 60 farenin gözüne enjekte edildi. 20 hafta sonra bu farelerin 10'u öldü, kalan 50 fare anestezi altında cerrahi olarak incelendi. Göz ve çevresinde hidatid kistin herhangi bir belirtisi yoktu. Kalan 39 fare 3 alt gruba ayrıldı ve 0.5 ml skoleks içeren solüsyon intraperitoneal olarak enjekte edildi. 20 hafta sonra, fareler anestezi altında açılarak periton, bağırsaklar ve karaciğer incelendi. Sonuçta, 6 farenin karaciğerinde Hidatid kist saptandı.

Anahtar Sözcükler: Göz, Karaciğer, Periton, Deneysel hidatik kist

INTRODUCTION

Echinococcus spp. are the cause of hydatid cyst (HC). It is a cosmopolitan parasite which causes a lot of economic and social problems (9). A lot of mammals are playing the role as intermediate hosts. Human is one of them. Infection is made by eating parasite eggs through the mouth. Protoscoleces are the infective stage of the parasite in the definitive host, but they are also able to differentiate asexually into secondary cysts when they are released through accidental rupture of the primary cyst (14). Most primary infections of *Echinococcus granulosus* in humans consist of a single HC, however, up to 20-40 percent of patients have multiple cysts or multiple organ involvement (11, 13). It can settle in different organs of liver (63%), lungs (25%), spleen, eye and other ones (13).

Hydatidosis is endemic in Iran and responsible for approximately 1% of admission to surgical wards (6, 8, 11). Considering the fact that HC can be ruptured easily so that protoscoleces can be spread all over the operating room including surgeons face and cloth, the personnel thought that the fluid of the cyst can cause ocular HC in them is a main problem in surgery rooms. It can be a source of anxiety to the personal of operating room (1).

Makale türü/Article type: **Araştırma / Original Research**
Geliş tarihi/Submission date: 26 Eylül/26 September 2009
Düzeltme tarihi/Revision date: 19 Ocak/19 January 2010
Kabul tarihi/Accepted date: 10 Şubat/10 February 2010
Yazışma /Correspoding Author: Khosrow Hazrati Tappeh
Tel: (+90) (232) 438 76 87 Fax: (+90) (232) 388 13 47
E-mail: hazrati_tappeh@yahoo.co.nz
This study was presented in 9th European Multicolloquium of Parasitology (2004, Valencia, Spain).

This work was supported by funding from the Research Center of Urmia University of Medical Sciences.

The personal think that it can cause ocular HC. During recent years, a lot of studies have been carried out in the forms of in vivo and in vitro on the *E.granulosus*, and so many experimental models have been done to make secondary HC (4).

In vitro cultivation of *E. granulosus*, frequently in vitro culture methods have been used in antigen production, vaccine and drug development (3).

In this study, Balb/c mice are infected by two ways: by spreading protoscolex solution into the mice eye and by injecting protoscolex solution into the mice peritoneum to produce HC.

If the eye case was positive, the researcher will try to suggest some treatment and prevention ways.

MATERIAL AND METHODS

Liver and lungs of infected sheep were collected from Urmia industrial slighter house and transferred to laboratory of parasitology department, Faculty of Medicine. The hydatid cyst fluid (HCF) was aspirated and examined for presence of protoscoleces. The viability of protoscoleces was determined by flame cell activity wit staining by 0.1% eosin (12).

Three different concentrations of protoscoleces, 500 protoscoleces/0.5 ml, 1000 protoscoleces/0.5 ml and 2000 protoscoleces/0.5 ml in physiological saline were prepared.

Balb/C mice maintained in the animal house of Faculty of Medicine were used for the study. The mice were divided into two groups, first and second groups having sixty and thirty nine, respectively. Two groups were divided to three sub groups (20 and 13 mice in subgroup one and two, respectively).

Every single mouse in the two groups was poured on the eye and injected intraperitoneal with protoscoleces as below:

First group (sub group A): 500 protoscoleces/0.5 ml eye

First group (sub group B): 1000 protoscoleces/0.5 ml eye

First group (sub group C): 2000 protoscoleces/0.5 ml eye

Second group (sub group A): 500 protoscoleces/0.5 ml intraperitoneal

Second group (sub group B): 1000 protoscoleces/0.5 ml intraperitoneal

Second group (sub group C): 2000 protoscoleces/0.5 ml intraperitoneal

After 20 weeks, the mice were examined for clinical symptoms and after anesthetize by diethyl-ether and their eyes were enucleated by surgical scissors and the location of the eyes were examined for HC. Peritoneum was examined in the second group.

RESULTS

From all the 60 mice in group one, 10 mice died before 20 weeks, with no clear reason. The 50 mice were examined regarding their blindness and proptosis.

In five cases of the first group (2 cases of subgroup A and 3 cases of subgroup C) eye proptosis were seen. After necropsy HC was not seen in the eye and around tissues of examined animals.

Eight mice from 39 cases in the second group were died before 20 weeks, with no clear reason. The peritoneum of the rest 32 mice were examined their liver and peritoneum. Hydatid cysts were seen in 6 (18.8%). The infected mice were belong to subgroup B (2 case) and subgroup C (4 case). There wasn't any HC were seen in 26 (81.2%) animals. The minimum and maximum cyst were isolated from infected mice were 2 and 13 respectively. The size of isolated cysts in liver and peritoneum were varied from 0.3 to 2 mm.

In this study, as it can be obtained from the results, in the second group, the best concentration to produce HC in the liver and peritoneum is a solution containing 2000 protoscolexces/0.5 ml.

DISCUSSION

Referring to human HC, it can be proved that the probability of eye HC occurrence is very low. In a study, it was showed during 19 years study only 18 (3%) eyes HC reported from 3736 HC cases. All the 18 cases had proptosis (2). Other studies also showed that the distribution of eye HC also is very low. Gomez *et al*., (5) reviewed 35 cases of orbital hydatid cyst, which represented 5% of orbital surgical cases from 1994 to 1985. Slowly progressive unilateral exophthalmoses, with or without pain, was the most frequent clinical manifestation.

Regarding the uncommon ways of getting HC such as dog biting, parasite egg or spreading HCF to the face and eyes of the operating room personal, it is thought that these people may get eye HC. None of experimental studies proved this factor up to now.

Producing HC in liver and peritoneum in the form of in vivo has a lot of research and treatment applications. Various experimental models have been proposed to produce HC in mice such as injecting protoscolex solution to the peritoneum or implanting daughter cyst in the Balb/c mice peritoneum (4).

Hokelek (2001), was examined two different experimental methods using mice. In one group daughter cysts with 0.5-1 cm diameter were removed from the large cyst and washed with sterile line. Two of these cysts were put into

the peritoneum cavity of each of 12 mice. Another 12 mice were injected intraperitoneally with 0.1 ml of a solution of protoscoleces that had been washed with normal saline. Three months later animals were sacrificed and the cyst sizes were compared. In group (A) the cyst which had been placed in the peritoneum had reached 1.5-2 mm diameter and in group (B) the largest intraperitoneally cyst was 3 mm in diameter (7).

In Breijo (1998) study, it is interesting to notice that miniature cysts were also recovered from mice infected with 1000 protoscoleces. These had a mean diameter of between 0.5 and 0.75 mm after 30 days of infection. The most dose for infection of mice was 1000 protoscoleces/0.2 ml (4). This amount in our study was 0.3 mm to 2mm. However, we observed growth rate in our model, when infection was done by using a dose of 2000 protoscoleces/0.5 ml. Thus, even thought the rate of parasite growth is probably determined by multiple factors (such as the origin and metabolic status of the protoscolex), the influence of the number of parasites inoculated on the growth rate should be studied.

Pennoit-De *et al*. (10), implanted daughter cysts by 1 mm diameter to the Balb/C mice. 9-14 months after implanting he opened the mice peritoneum to examine implanted cysts. In a month, the mice have an average over weight of 0.3±0.03mg. In general, the cysts have 0.27±0.02 mg overweight. In our study, the cysts size reached to 2 mm. Hydatid cyst can be produced by different ways in intraperitoneum and liver. It usually reachs to the suitable growth and size, so to treat HC, different animal models can be used.

REFERENCES

1. **Akoglu M, Davidson BR**, 1992. A rational approach to the terminology of hydatid disease of the liver. *J infect*, 24(1): 1-6
2. **An Xiao, Cheng Xueyi**, 1999. Hydatid cysts of the orbit in xinjiang are view of 18 cases. *Orbit,*18(3): 151-155.
3. **Bolukbas CS, Doganay A**, 2008. In vitro cultivation of *Echinococcus granulosus*. *Turkiye Parazitol Derg*, 32(4): 360-365.
4. **Breijo M, spinelli P, Sim RB, Ferreira AM,** 1998. Echinococcus granulosus: An intraperitoneal diffusion chamber model of secondary infection in mice. *Exp parasitology*, 90(3): 270-276.
5. **Gomez Moralis A, Croxatto JO, Crovettol, Ebner R**, 1988. Hydatid cysts of the orbit. A review of 35 cases. *Ophthalmology*, 95(8): 1027-1032.
6. **Hadighi R, Mirhadi F, Rokni M**, 2003. Evaluation of a dot-ELISA for the serodiagnosis of human hydatid disease. *Pak J Med Sci*, 19(4): 268-271.
7. **Hokelek M, Erzurumlu K, Uyar Y, Guvenli A**, 2001. Comparison of Two Methods of Experimental Cystic Echinococcosis in BALB/C Mice. *Turkiye Parazitol Derg*, 25(2): 142-144.
8. **Lotfi M**, 1999. Hydatid cyst diseases. 1st ed. Tehran, Iran. Sahab Press.
9. **Muller R,** 2002. Worms and human diseases. 2 nd ed. Wallingford: CABI International, Oxon, UK.
10. **Pennoit –De Cooman E, De Rycke PH**, 1978. Serial passages of larval Echinococcus granulosus from equine origin in mice. II. infections with sterile cysts. *Z Parasitenkd*, 56(1): 39-45.
11. **Rokni MB**, 2009. Echinococcosis/hydatidosis in Iran. *Iranian Journal of Parasitology*, 4(2): 1-16.
12. **Smyth JD, Barret NJ**, 1980. Procedures for testing the viability of human hydatid cyst following surgical removal, especially after chemotherapy. *Trans R Soc Trop Med Hyg*, 74: 649-652.
13. **Topley K, Wilson S**, 2005. Microbiology and microbial infections PARASITOLOGY, 10th ed, Hodder Arnold, London: 677-712.
14. **Thompson RCA,** 1995. Biology and systematics of Echinococcus. Chapter 1, In *Echinococcus and Hydatid Disease* (R.C.A. Thompson and A. J. Lymbery, Eds), pp. 1-37. CAB International, Oxford shire, UK.