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KADİR KAZEZ

MUSTAFA ŞAHİN

CELALETTİN VATANSEV

YÜCEL ARITAŞ

FARUK AKSOY

See next page for additional authors

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Authors

KADİR KAZEZ, MUSTAFA ŞAHİN, CELALETTİN VATANSEV, YÜCEL ARITAŞ, FARUK AKSOY, and HÜSAMETTİN VATANSEV

Kadir KAZEZ¹
Mustafa ŞAHİN²
Celalettin VATANSEV²
Yücel ARITAŞ³
Faruk AKSOY²
Hüsametttin VATANSEV⁴

Experimentally Developed Secondary Echinococcosis in Pleural and Peritoneal Cavities and the Utility of Serological Tests During the Follow up*

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Abstract:

Secondary echinococcosis is an important complication of hydatid disease and is not rare in the peritoneal cavity. However, the frequency of secondary hydatidosis in the pleural cavity is not very well known.

This study was planned to elicit the frequency of secondary cyst development in the pleural cavity, and to compare this with secondary peritoneal hydatidosis. It was also intended to follow up cyst development by serological tests.

Twelve white female Island rabbits were used. The mean weight of the rabbits was 2450±420 g. Cystic fluid containing protoscolex, hydatid sand and germinative membrane homogenate was injected via an 18 F needle into the pleural and peritoneal cavities of the rabbits. At the beginning and the fourth and fifteenth weeks of the study, 5 cc of blood was withdrawn from the ear veins of the rabbits for serological investigations. All rabbits were sacrificed by cervical dislocation at the end of the fifteenth

week of the study. Peritoneal and pleural cavities were opened and examined for secondary cyst development. Serological investigations were performed with indirect hemagglutination and indirect fluorescein antibody tests.

The frequency of secondary echinococcosis in the peritoneal cavity was 80% and, in the pleural cavity 30 %. In determining the antibodies against the hydatid antigens the indirect fluorescein antibody test was more sensitive than the indirect hemagglutination test.

The occurrence of secondary echinococcosis is possible in the pleural cavity, but to a lesser degree than in the peritoneal cavity. Indirect hemagglutination and indirect fluorescein antibody tests were found to be valuable serological tools for the diagnosis of hydatid disease.

Hydatid cyst, secondary echinococcosis, pleural and peritoneal cavities, IHA and IFA tests.

¹Department of General Surgery, State Hospital, Elazığ, Departments of ²General Surgery, ⁴Biochemistry, Faculty of Medicine, Selçuk University, Konya, ³Department of General Surgery, Faculty of Medicine, Erciyes University, Kayser-Turkey

Introduction

Although cystic hydatid disease has been entirely eradicated in some countries, it remains a serious health problem in certain parts of the world. It is endemic in the Middle East and the Balcanic countries including Turkey. Spread of cystic hydatid disease can be attributed to continual migration.

Man is an incidental intermediate host, inseminated by the parasite frequently through the ingestion of the ova and rarely by inhalation. The hexacanth embryo becomes free after digestion of the ova in the gastrointestinal tract and the resultant embryo most often settles down in the liver via the portal circulation. The embryo, which passes through the liver and crosses to the lymphatic system,

then enters the systemic circulation and may involve various organs where it can cause cystic hydatid disease(1).

Germinative membrane and scolex have the potential of new or secondary cyst formation (1-3). The most important and occasional complication of the hydatid cyst is spontaneous, traumatic, or operative rupture into the pleural or peritoneal cavity to seed for secondary echinococcosis (4-11).

Secondary echinococcosis is not rare in the peritoneal cavity after the rupture of a primary cyst such as liver, spleen, kidney or intra-abdominally located hydatid cysts, usually resulting in secondary echinococcosis in the peritoneal cavity (1, 4, 7, 11-13). However, the

* This study was carried out at Erciyes University Medical Research Center, Kayseri, Turkey.

frequency and the mechanism of secondary hydatidosis in the pleural cavity is not very well known. There are many controversial findings and reports about this event (1, 14-17).

This study was designed and carried out to determine whether a secondary cyst can develop in the pleural cavity, and if so, to determine its frequency and to compare it with peritoneal hydatidosis. It was also intended to utilise serological tests to follow up cyst development in the experimental animals.

Materials and Methods

Twelve white female island rabbits were used in this study. The mean weight of the rabbits was 2450±420 g.

Hydatid cyst containing sheep livers were obtained from the Kayseri Meat Institute. Cystic fluid was collected by aspiration of the cysts under aseptic conditions. In order to increase the amount of scolex, the fluid was collected into a 10 cc syringe by back and forth aspiration. This fluid was put into a sterile centrifuge tube for 10 minutes. Scoleces precipitated at the bottom of the tubes and the supernatant fluid was discharged. A drop of hydatid sand including scoleces was taken with a sterile pasteur pipette to determine the viability of protoscoleces under a light microscope (1, 2). Germinative membrane and hydatid sand of the cysts were obtained under sterile conditions, and prepared by homogenizing to a density which could be injected.

After the right hemithorax and the abdominal wall of the rabbits were shaved, 5 cc of blood was withdrawn from the ear veins and saved for serological tests. 0.5 ml of fluid containing protoscolex and germinative membrane and hydatid cyst formation was injected separately, via an 18F needle into the pleural and peritoneal cavities of the rabbits. After inoculation, the rabbits were left in their cages for 15 weeks.

At the fourth and fifteenth weeks of the study, 5 cc of blood was withdrawn from the ear veins of the rabbits for serological investigations. At the fifteenth week of the

study, all rabbits were sacrificed by cervical dislocation. The peritoneal and pleural cavities were opened with a midline laparotomy and sternotomy. The cavities were examined carefully for hydatid cyst formation. The number of cysts, their size and location were determined and recorded. After photographs were taken, all the cysts were excised and kept in 10 % formaline solution for histopathological examination.

Serological investigations were performed with indirect hemagglutination (IHA) and indirect fluorescein antibody (IFA) tests. In the IHA test, the patient's sera is mixed with sensitive red blood cells in different titrations in a tube. In the presence of antibody in the sera, irregular precipitation of the sensitive red blood cells occurs. In the absence of antibody, the red blood cells neatly precipitate at the bottom of the tube (18). In the IFA test, antibodies in the patient's sera bind to the antigen particles. This complex binds to the antihuman gammaglobulin that is marked with florescein isothiocyanate (18, 19).

Paraffin blocks of the tissue samples were prepared, stained with haematoxylin-eosin and examined under a light microscope. Microscopic pictures were taken when necessary.

Student t test was used for statistical analysis. A p value of <0.05 was accepted as statistically significant.

Results

Two rabbits died after injection of the cystic fluid and were excluded from this study, which, therefore, was completed with ten rabbits.

Secondary echinococcosis developed in the peritoneal cavities of eight rabbits and in the pleural cavities of three rabbits (p<0.05). There was no cyst formation in the peritoneal and pleural cavities of two of the rabbits. The diameters of the cysts varied between 2 and 8 mm. In total, there were 39 cysts in the peritoneal cavities of eight rabbits and 7 cysts in the pleural cavities of three of the rabbits (Table 1).

Table 1. Number of cyst in the pleural and peritoneal cavities.

Rabbits No:	1	2	3	4	5	6	7	8	9	10
Cyst Numbers:										
Peritoneal Cavity	3	4	4	6	5	3	6	-	8	-
Pleural Cavity	-	-	-	2	2	-	-	-	3	-

Adhesions were noticed in the pleural and peritoneal cavities of all rabbits, around the injection sites. All of the peritoneal cysts were located on the intestinal surface and the parietal peritoneum overlying the abdominal wall which was punctured with the needle (Figure 1). Pleural cysts were located on the lower parts of the right lungs (Figure 2).

There was no positive result with the IHA and IFA tests in the blood sera of the rabbits withdrawn before the study. On the basis of these results it was decided that the rabbits did not have hydatid cysts, or any disease likely to have cross-reaction with a hydatid cyst, before the study.

Positive results were detected in 1/4 and 1/16 of the titrations with the IHA test in the blood sera withdrawn at the end of the fourth week. There was no antibody measurement in any titration in the blood sera of the two rabbits without cysts. In the blood sera withdrawn at the fifteenth week, antibody measurements showed positive results in 1/4 and 1/32 titrations in all animals. Antibody titrations correlated with the number of the cysts and cyst duration (Table 2).

In the blood sera of the rabbits that was withdrawn at the fourth week of the study, positive results were achieved at 1/4 and 1/32 titrations with the IFA test in all animals. Positive results were measured at 1/4 and 1/128 titrations in the blood sera of all animals at the end of the fifteenth week (Table 3).

Although there was no antibody measurement with the IHA test in the blood sera of two cyst-free animals at the fourth week, positive results were achieved with the

IFA test in the same blood samples. It was also noticed that the IFA test gave positive results in lower titrations than the IHA test. In spite there being of no cyst formation, positive results were thought to be due to the injected cystic content.

For comparison in determining the antibodies against the hydatid antigens, the IFA test was found to be more sensitive than the IHA test. The titrations that were measured with the two tests showed correlation in each animal.

On histopathological examination, the laminar layer and germinative membrane were seen in all cysts (Figure 3).

Discussion

Secondary echinococcosis is a severe complication of hydatid disease. Although spontaneous or traumatic cyst rupture and the resultant peritoneal spillage of cystic contents can cause cyst inoculation, the main cause of secondary cyst development is due to operative spillage of the scoleces (1, 9, 15).

When the scoleces, which have the ability of reproduction and asexual growth, spread, they are usually eradicated by host defense mechanisms. However, the scoleces that remain viable can develop secondary cysts months or even years later(1).

The incidence of secondary cyst development is not known for certain. Mottaghian (12) found out that the incidence of secondary cyst development in his patients, followed up for 3 to 8 years, was 11.3%. Morris (20)

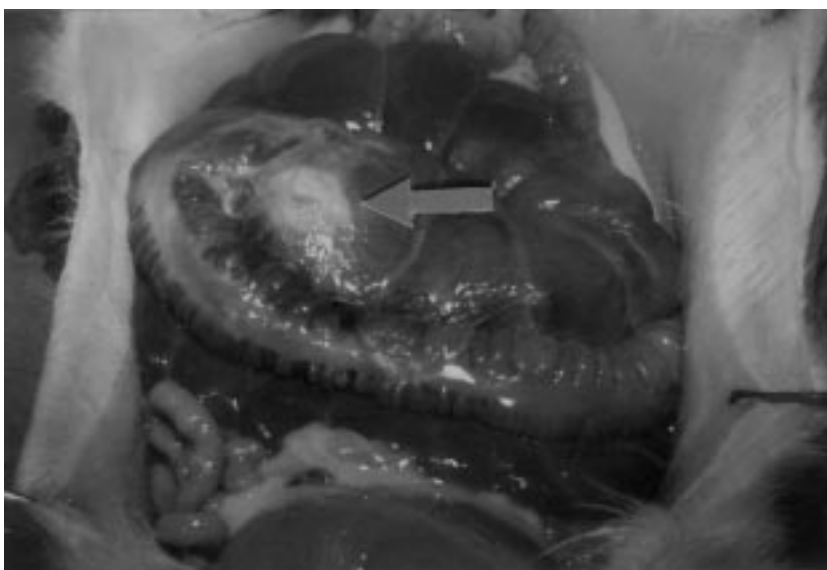


Figure 1. Appearance of a peritoneal cyst at fifteenth week. Cysts located on the surface of the intestinal peritoneum.

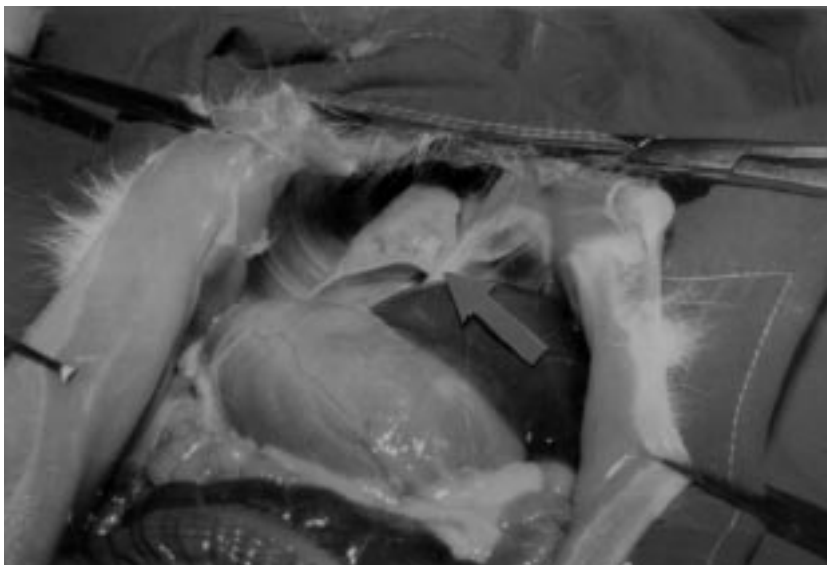


Figure 2. Appearance of a pleural cyst at the fifteenth week. All cysts located on the lower parts of the lungs.

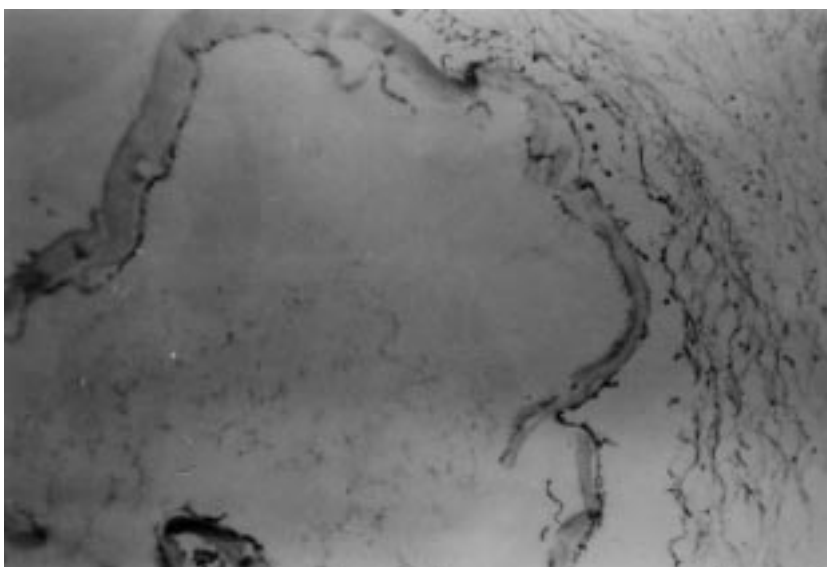


Figure 3. Microscopic appearance of a cross section of a cyst. One layer of germinative membrane and laminar layer is evident (stained haematoxylin – eosin. X 36).

noted that the frequency of secondary echinococcosis was 10% in general, but this was 30% in peritoneal hydatidosis.

The host defense mechanisms, patient's age, viability, the number of spilled scoleces and serosal surfaces were accepted as the most important factors in the development of secondary echinococcosis (1, 4, 12, 21). Although serosal surfaces are suitable places for the adherence and growth of scoleces, it has been suggested that mucosal surfaces are not suitable places for the nourishment of scoleces (12). Rupture of cysts into the bronchus or biliary tract does not lead to development of

secondary cysts. This is the most important evidence supporting the idea that mucosal surfaces are not suitable for formation of the secondary echinococcosis (4). Peritoneal surfaces that have been exposed are a good environment for the development of secondary echinococcosis (1, 2, 4, 7, 12, 22). The incidence of peritoneal cyst development was found to be 80% in this study. This result supports the idea that the peritoneal cavity is a good environment for secondary hydatidosis.

On the other hand, there are contradictory findings and opinions regarding the development of secondary cysts in the pleural cavity. While several authors accept

Table 2. Antibody titrations measured by indirect hemagglutination test.

Rabbits No:	1	2	3	4	5	6	7	8	9	10
Fourth week	1/8	1/4	1/4	1/8	1/6	1/8	1/8	-	1/8	-
Fifteenth week	1/8	1/8	1/6	1/8	1/32	1/16	1/32	1/4	1/8	1/8

Table 3. Antibody titrations measured by indirect florescein antibody test.

Rabbits No:	1	2	3	4	5	6	7	8	9	10
Fourth week	1/8	1/4	1/16	1/8	1/32	1/32	1/8	1/4	1/16	1/8
Fifteenth week	1/16	1/8	1/128	1/8	1/128	1/128	1/16	1/8	1/16	1/16

the possibility of secondary echinococcosis in the pleural cavity, others dispute this (1, 12, 15-17, 22, 23).

Authors such as Dew (24), Hankins (25), and Yalav (16) have suggested that cysts that rupture into the peritoneal cavity can develop secondary cysts 2-10 years later, but that scoleces which spread to the pleural cavity can not develop a new cyst. Contrary to these authors, Saidi (1), Mottaghian (12), Schiller (26) and Lewal (4) reported that, in spite of some negativeness, secondary cysts can develop in the pleural cavity. When a hydatid cyst ruptured into the pleural cavity, serious clinical conditions such as pneumothorax, pyopneumothorax or empyema can develop. These are life threatening situations which require immediate diagnosis and treatment to reduce the likelihood of secondary cyst development (12, 23, 27, 28). We found 30% cyst development in the pleural cavity. On the basis of this finding, we propose that secondary hydatid cyst development in the pleural cavity is possible, although, it is not a good environment for cyst development.

Saidi (1) suggested that the cysts which were opened to the pleural cavity should be regarded as two different types according to their origin, the liver or the lung. He claims that, liver cysts are usually multivesicular and when they are opened to the pleural cavity, they can develop secondary cysts. Contrary to this, lung cysts are univesicular, and when they rupture into the pleural cavity, they can cause severe clinical pathologies such as pneumothorax, peyopneumothorax and abscesses. These situations prevent the development of secondary cysts in the pleural cavity (17, 29). Although we excluded these types of pathologies by injection of hydatid sand via a needle, the degree of cyst development in the pleural

cavity is lower than in the peritoneal cavity. These results suggested that other factors may be important in reducing the number of secondary cysts in the pleural cavity. Friction of pleural sheets during respiration might be an important factor in preventing the scoleces adhering to the pleural surface, and hence, in developing new cysts. In this study, the cysts were located on the lower parts of the lungs, which showed the least expansion and friction during respiration. This observation supports our conclusion.

Several serological tests have been used in the diagnosis and follow-up of the development of hydatid disease. The IHA and IFA tests are well-known, sensitive and commonly used techniques in many laboratories (18, 19, 30, 31).

At the beginning of the study, antibody could not be exposed in the sera of the rabbits with each test. Hence, it was thought that, the experimental animals were not carrying hydatid disease or another disease giving cross reaction with echinococcosis. Both tests gave positive results correlating with the cyst number, cyst size and cyst duration at the fourth and fifteenth weeks of the study. The measurement of antibodies in lower concentrations with the IFA test suggests that the IFA test is more sensitive than the IHA test.

However, the presence of antibodies in the sera of two rabbits without viable cyst is an interesting finding. This may be due to antibodies developed against cystic material injected at the beginning of the study. On the basis of this assumption, we propose that antibodies can be detected in the sera of a patient after the treatment of hydatid cyst until the cystic material disappears. Positive results, after the treatment of a cyst, can cause confusion

at follow-up about if the cyst is treated successfully, whether the presence of another cyst has been overlooked or early relapse has occurred.

In conclusion, secondary cyst development was shown in a frequency of 80% in the peritoneal cavity and 30% in the pleural cavity of the rabbits, by external inoculation in this experimental study. The development of fewer cysts in the pleural cavity supports the idea that this cavity is not a good environment for secondary echinococcosis. However, it should be kept in mind that secondary echinococcosis is possible in the pleural cavity. The less frequent occurrences suggest that respiration may prevent the adhering to adhere to the pleural surface, thereby reducing their nourishment capacity. Technical difficulties during the injection of hydatid fluid into the thorax might be another factor preventing cyst development in the

pleural cavity. In contrast, it is clear that the peritoneal cavity is a good environment for the development of secondary hydatid cysts.

The IHA and IFA tests gave positive results which correlated to the cyst number, cyst size and cyst progression. Hence, these two tests were found to be valuable serological tools for the diagnosis of hydatid disease and follow-up after treatment. They can also be used as screening tests for echinococcosis in endemic fields.

Correspondence author:

Mustafa ŞAHİN

Selçuk University Medical Faculty

Department of General Surgery

42080 Meram-Konya-TURKEY

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