Diagnosis of secondary hydatid cysts in white mice by ultrasonography and Doppler examination

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SUMMARY

In this study, ultrasonography controls of the secondary hydatid cyst of mice infected with sheep and donkey origin protoscolecetes of the hydatid cyst. In the ultrasonographic examination, no problem was encountered with the diagnosis of both control and infected groups. Furthermore, in the Doppler examinations of the infected and control groups, statistically significant associations were found between Resistive Index (RI) and Pulsative Index (PI) values (at p<0.001).

Experimental hydatid cyst formation via inoculation of laboratory animals with Echinococcus protoscolecetes is a widely used technique. Because it eliminates the dangers of accidental infection risks during working with Echinococcus eggs. It is easy and inexpensive to maintain rodents and it enables researcher to work with large numbers of animals. However, one of the disadvantages of the technique is that, the infected mice have to be killed in order to monitor the development of infection progress. The purpose of this study is to determine the possible role of ultrasonography and color Doppler in the observation of the cysts.

According to the results of the study, it was seen that ultrasonography and color Doppler could be used in the detection and localization of the cysts. It is also concluded that, no differentiation between secondary cyst hydatid infections induced by protoscolecetes from different host species could be made by ultrasonographic or color Doppler examinations.

Keywords : Diagnosis - Doppler - Echinococcus granulosus - hydatid cysts - mice - ultrasonography.

Introduction

Echinococcosis, important to both human and animal health, is a parasitic disease seen in almost every society, especially in underdeveloped and developing countries where environmental health and preventive medical measures are inadequate. Echinococcus granulosus granulosus parasitizes in the small intestines of most canines except the fox whilst E. granulosus equinus is found in the small intestines of all canines including the red fox [5, 9, 19, 20, 22].

In humans and animals, the diagnosis of hydatid cysts is based on the clinical symptoms as well as the radiographic and ultrasonographic findings and serological test results [1, 4, 6, 11, 13, 15, 21]. In ultrasonographic examination, the hydatid cysts are seen as anechoic areas with most having internally localized round septa [2, 3, 7, 8, 10, 12]. Cysts are seen as either single or multiple inclusions which lead to enlargement of the organs in which they are found [2, 7]. When the cysts are located in the liver tissue they cause lesions in the liver vessels which in turn may lead to formation of intrahepatic and extrahepatic shunts. Changes in the blood flow rate occur due to all these changes [14, 16, 17]. In some hydatid cysts, formation of pseudo-membranes which gives them a honey-comb appearance have been described. On occasion, calcification of the hydatid cysts have been described [2, 3, 8].

In veterinary radiology, Doppler ultrasonography is a rather new technique with increasing applications. With this method, parameters such as the rate of blood flow and its direction in the vessels of organs can be determined [14, 16, 17, 18]. The image in Doppler ultrasonography provides qualitative data on blood flow. For this purpose, it is used in combination with double imaging techniques that give graphical doppler spectra, instead of using it alone [18]. With the qualitative data, the presence of blood flow, its direction of flow and its characteristics (e.g. monophasic, biphasic, triphasic) can be determined. In the double imaging techniques however, data on both the flow rate and vessel resistances can be measured [14, 16, 17, 18]. With Doppler ultrasonography, the hepatic vascular structures and changes in

RéSUMÉ

Diagnostic des kystes hydatiques chez les souris blanches par échographie et examen Doppler. Par O. SARIMEHMETOGLU, A. BUMIN ET B. GÖNENÇ.

Dans cette étude, l’échographie a été utilisée pour détecter la présence de kystes hydatiques chez des souris infestées avec des protozoïdes provenant de moutons et d’ânes. Au cours de ces examens, aucune difficulté n’a été rencontrée, tant chez les animaux sains que chez les animaux infestés. L’obtention expérimentale de kystes hydatiques après infestation d’animaux de laboratoire avec des protozoïdes d’Echinococcus est une technique largement utilisée, limitant les dangers de travailler directement avec des œufs d’Echinococcus. De plus, il est facile et peu coûteux de maintenir des rongeurs ce qui permet au chercheur de travailler avec un grand nombre d’animaux. Cependant, un des inconvénients de la technique est la nécessité de sacrifier des souris infestées afin de surveiller le développement de l’infection. Le but de cette étude est de déterminer l’aide de l’échographie et de l’examen Doppler dans l’observation des kystes.

Selon les résultats de l’étude, ces deux techniques peuvent être utilisées dans la détection et la localisation des kystes. Ces deux techniques ne permettent pas de différencier l’espèce d’origine des protozoïdes infestants.

these structures can be visualized [18].

Experimental hydatid cyst formation via inoculation of laboratory animals with *Echinococcus* protoscoleces is a widely utilized technique because it eliminates the dangers of accidental infection risks during working with *Echinococcus* eggs. It is easy and inexpensive to maintain rodents and it enables researcher to work with large numbers of animals. However, one of the disadvantage of the technique is that, the infected mice have to be killed in order to monitor the development of infection progress. In order to overcome the above mentioned problem, this study aimed to determine the possible role of ultrasonography and color Doppler in the observation of the cysts.

**Materials and methods**

In the study, 4-5 week-old male and female white mice (Swiss albino) were used. All mice were raised in our department. They were grouped and kept in transparent macrolent cages of 43x27x15cm according to the origin of the infection and their genders, and fed with commercial feeds. Of these mice, 8 were infected experimentally with 4,000 hydatid cyst protoscolexes of sheep origin and 7 mice infected with protoscoleces of donkey origin. Fifteen mice were also selected to represent the control group. After infection, the mice were kept for a period of 10 months before ultrasonographic and doppler ultrasonographic examinations were conducted by a veterinary radiologist blinded to the control and infected groups.

For the ultrasonographic and color Doppler examinations, an ESAOTE brand AU5 model Doppler ultrasonography device with a 10 mHz linear probe was employed. The abdominal skin was shaved with the aid of a gel, the probe was moved over the area in the transverse and longitudinal planes for the examination. Firstly, portal vessels of the liver were indentified by color Doppler ultrasonographic examination. Then, hepatic artery was found by slightly deviated the probe to the medial. By taking blood flow speed spectrum from hepatic artery Resistive Index (RI) and Pulsative Index (PI) values were measured.

After the ultrasonographic and Doppler examinations, the mice were killed humanely with ether, and autopsies were performed to confirm the presence of hydatid cysts their numbers and sizes. The results obtained from autopsy were compared with those from the ultrasonographic studies.

The blood flow mice in the infected and control groups were measured with the Doppler technique. RI and PI values obtained from the measurement were evaluated by comparing the control group to the infected group with the independent sample student t-test.

**Results**

In the ultrasonographic examination of the infected mice under the B-mode gray scale, varying numbers of cysts were determined in all cases. The cysts were found to have internal anechoic contents surrounded by a hyperechogenic capsule (Figure 1). Most of the cysts were found to be located within the abdominal space while those located within organs were all found within the liver tissues. Autopsies performed after the diagnostic studies revealed between one and 70 cysts in the mice. No protoscoleces were encountered in the cysts. While the smallest of the cysts measured 0.1 x 0.1 cm, the largest measured 2 x 1.5 cm. The cases in which 5-6 cysts with varying sizes were found by the ultrasonographic examination were observed correspondingly with the autopsy findings. However, in those found to have more than 5-6 cysts, there were great differences between the number found at autopsy and that determined by ultrasonography. While in some cases with higher cyst counts the ultrasonographic examination showed a honey comb pattern, in 4 cases, cysts with the rather typical hyperechogenic double capsules were found (Figure 2). An anechoic area was found between the two capsules under ultrasonography. The blinded radiology specialist who made the diagnosis of the hydatid cysts in the experimentally infected mice did not observe any distinguishing features between the protoscoleces of sheep origin and those of donkey origin.

In the Doppler ultrasonographic study, the blood flow rates of the intrahepatic blood vessels of the liver were determined qualitatively. Later, the quantitative blood flow rates of these intrahepatic vessels were determined by measuring the RI and the PI from the hepatic artery with the duplex Doppler ultrasonography (Figure 3). In the B-mode ultrasonographic examination of the control group mice, no abdominal pathology was observed. In these mice the RI and PI values from the intrahepatic hepatic artery were also measured. The RI and PI values of the infected group was found to be significantly higher than that of the control group (p<0,001- table I).

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>X ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistive Index (RI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected</td>
<td>15</td>
<td>0.69 ± 0.04</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>0.53 ± 0.2</td>
</tr>
<tr>
<td>Pulsative Index (PI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected</td>
<td>15</td>
<td>1.84 ± 0.1</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>1.44 ± 0.03</td>
</tr>
</tbody>
</table>

Table 1.—Statistical results of resistive and pulsative indexes of infected and control group.

* = significant difference relative to the infected group, p<0,001.
Hydatid cysts of various shapes have been reported to appear as cysts with round anechoic central cores surrounded by a fibrous capsule in varying numbers under ultrasonography of the host (human) [2, 3, 6, 8]. In another study conducted similarly, the B-mode gray scale ultrasonographic examination of infected mice demonstrated varying numbers of cysts in all cases. The cysts appear anechoic because they are filled with fluid. Also, due to the markedly fibrous capsule formation, the periphery appear hyperechogenic. In cases with fewer cyst numbers, a more echogenic appearance was seen compared to those with much higher cyst numbers due to the fact that the capsule was more developed.

Cysts are developed either monofocally or multifocally. At the affected organs, the enlargement was low if monofocal, and high if multifocal [2, 3, 8]. Though majority of the cysts in the infected mice were located within the abdominal space, all those found under ultrasonographic studies to be located within organs were found in the liver parenchyme. These results were confirmed by autopsy examinations performed later. In the ultrasonographic examination of the mono-cysted cases the lose of the appearance in the liver paranchym tissue was not observed. However, as the number of cyst increased, the homogeneous echo-structure of the liver parenchyme tissues have begun to change to heterogeneous structure. Furthermore, there was significant difference in the liver enlargement between the mice with single cysts and those with multiple cysts.

It has been reported that accurate estimate of the number of cysts by ultrasonographic examination becomes more difficult with increasing cyst numbers [8]. During the ultrasonographic examinations, of the infected mice, except one case, more than one cyst were found. There was a correlation between the ultrasonographic examination and necropsy findings at the case which have 5-6 cysts. In mice found to have over 6 cysts, however, the number determined by ultrasonography showed significant difference with those obtained by autopsy. An explanation for this is the fact that multiple cysts have a tendency to interfere with each other’s image under ultrasonography.

In cases with greater cyst numbers, the cysts were seen as multicysts with honey comb pattern under ultrasonography. In some cases they were seen with double margins due to the germinative layer. In such situations, the germinative layer were seen protruding into the cystic space which has been described as being pathognomonic for the diagnosis of hydatid cysts. It has been reported that, with the protrusion, so formed a fall in the intracystic pressure results leading to separation of the two membranes [2, 8]. In a similar manner, in cases with more cyst numbers, alignment of the fibrous membranes in a contiguous fashion results in the honey comb appearance. In the ultrasonographic examination, 4 cases with the appearance of double epithelium originating from the germinative epithelium were identified. An anechoic area was observed between the external fibrous capsule and the germinative epithelium under ultrasonography as a result of the collapse of the germinative epithelium and hence a fall in the intracystic pressure. It has been reported that some old hydatid cysts can become calcified [8]. Calcification was not encountered in any of our cases, however.

In this study, evaluation of the symptoms caused by the secondary cyst hydatid in white mice with ultrasound and color Doppler ultrasonography was examined. According to the results of the study, it was seen that ultrasonography and color Doppler could be used in the detection and localization of the cysts. It is also concluded that, no differentiation between secondary cyst hydatid infections induced by protoscoleces from different host species could be made by ultrasonographic or color Doppler examination.
References


