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Mesenteric Doppler Ultrasonography Findings of *Echinococcus multilocularis* Infection: An Experimental Study

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SUMMARY: The diagnosis of alveolar echinococcosis, caused by the larval stage of *Echinococcus multilocularis*, is often difficult and almost always possible only in the later stages of the disease. In this study, we aimed to evaluate the findings of Doppler ultrasonography images for the diagnosis of intraabdominal non-visceral alveolar echinococcosis in *Meriones unguiculatus*. Six infected animals were studied. Abdominal Doppler ultrasonography examinations were performed 20-25 days after the implantation. Then, animals were sacrificed and infected specimens were histopathologically examined. The sonographic examinations of the infected animals revealed lobulated, heterogeneous cystic intraabdominal masses. There were echogenic solid areas with hypoechoic and anechoic cystic areas within the lesions. Doppler ultrasound examination revealed vascular islands within those heterogeneous cystic lesions. Color mode showed multiple vascular coding areas within the solid part of the lesions, most of them were venous structures but there were also arterial vessels showing dominant flow with low resistive indices. Vascular structures were supposed to be the invaded native mesenteric vessels within the infiltrating inflammatory mass lesions. We consider that the Doppler ultrasound finding of irregular mesenteric vascular structures within intraabdominal heterogenous mass lesions may be an important sign for the diagnosis of experimental non-visceral alveolar echinococcosis in *Meriones unguiculatus*.

Key Words: Alveolar echinococcosis, diagnosis, Doppler ultrasonography

Echinococcus multilocularis Enfeksiyonunun Mezenterik Doppler Ultrason Bulguları: Deneysel Çalışma

ÖZET: *Echinococcus multilocularis* (Em)'in larvası tarafından oluşturulan alveolar ekinokokkoz 'un tanısı sıklıkla zordur ve hemen hemen daima hastalığın ileri evrelerinde olasıdır. Bu çalışmada; gerbillerdeki karın içi organ dışı alveolar echinococcosis enfeksiyonunun tanısında Doppler ultrasonografi görüntüleme bulgularını değerlendirmeyi amaçladık. Hastalığın hayvan modeli *Meriones unguiculatus* türü gerbillerde geliştirildi. Altı enfekte hayvan üzerinde çalışıldı. Doppler ultrasonografi dahil abdominal ultrasonografi implantasyondan sonraki 20-25. günlerde uygulandı. Daha sonra hayvanlar sakrifiye edilip enfekte örnekler histopatolojik olarak incelendi. Enfekte hayvanların ultrasonografik incelemesi, karın içinde lobule, heterojen kistik lezyonları ortaya koydu. Lezyonlarda hipoekoik ve anekoik kistik bölgeleri olan ekojenik solid kısımlar mevcuttu. Doppler ultrason incelemesi, heterojen kistik lezyonlarda vasküler adaları gösterdi. Renk modu, lezyonların solid kısımlarında multipl vasküler kodlanmayı ortaya koydu. Bunların çoğu venöz yapılardı ancak aynı zamanda düşük direnç indeksleri olan dominant akım gösteren arteriyel damarlar da vardı. Vasküler yapıların, infiltran inflamatuar kitle lezyonlarında doğal mezenterik damarları invazyona uğrattığı düşünüldü. Deneysel olarak *Meriones unguiculatus* türü gerbillerde, karın içindeki heterojen kitle lezyonları içinde düzensiz mezenterik vasküler yapıların doppler ultrason bulgusunun, organ dışı *E. multilocularis* enfeksiyonu için önemli bir tanısal bulgu olabileceğini düşünüyoruz

Anahtar Sözcükler: Alveolar echinococcosis, tanı, Doppler ultrasonografi

INTRODUCTION

The larval stage of the small fox tapeworm *Echinococcus multilocularis* (Em), a parasite prevalent in the Northern Hemisphere, is the causative agent of human alveolar echinococcosis, which is considered to be the most lethal helminthic infection in humans (10). Traditional radiological imaging methods can be used to diagnose infection and inflammatory processes by detecting the results of anatomical changes, but in the early phase of the disease, anatomical changes may be insufficient for the diagnosis. Alveolar echinococcosis usually appears on ultrasound (US) as a mosaic mixture of various echo patterns such as granular strong echo with or without acoustic shadow, irregular echogenic areas, small hypoechoic, and large anechoic or hypoechoic areas. The diagnosis of Em

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infection by US is sometimes difficult because of its nonspecific imaging findings (11, 12). Furthermore, radiological methods usually display image of a visceral organ, not the whole body, especially intraabdominal mesenteric structures.

The aim of this experimental study is to demonstrate the imaging characteristics of intraabdominal non-visceral lesions of Em infection with US and Doppler US in an experimental animal model.

MATERIAL AND METHODS

The Local Ethics Committee of the Experimental Studies at our university school approved the study. All animals were anesthetized with ether during the model preparation, imaging processes and while sacrificing.

Selection of animals and preparation of surgical implantation material: Em metacestodes were taken from stock infection in Mongolian gerbils (*Meriones unguiculatus*) and were grafted intraperitoneally into 3-month-old gerbils. Twenty-five days after infection, gerbils were sacrificed by cervical dislocation, and metacestodes (which appeared as cysts) were collected, cleared from the host tissue, and washed thoroughly in 0.9% sodium chloride as previously described (7, 9).

Six healthy female, 8-12 weeks old *M. unguiculatus* (approximately 60-70 gr.) were selected for the study. The exclusion criterion from the research was determined to be the presence of an infection or systemic reaction.

Tissue blocks of Em vesicles with a volume of 1 cm^3 were cut into pieces very carefully, with a sterile scalpel and then were placed in a Petri dish under the sterile conditions for each animal. The tissue in the Petri dish was washed three times with physiological saline. The infected tissues were placed into the abdominal cavity of the uninfected animals operatively under the previously mentioned conditions (4, 5). The success of implantation was confirmed by the palpation of the masses in the abdomen within 20-25 days.

Ultrasonography and Doppler Ultrasonography: All of the animals were examined by the Doppler US on the 20th-25th days after implantation. Abdominal ultrasonographic and Doppler ultrasonographic examinations were performed by a linear probe (5-12 MHz, ATL- HDI 5000, Bothell, Washington, USA) with the superficial imaging algorithm. Images were obtained on the transverse and longitudinal planes and spectral Doppler measurements were documented.

After the imaging, all of the infected animals were anesthetized with ether and sacrificed by cervical dislocation. At the end, an abdominal incision of 2-3 cm was performed in the midline of the sacrified animals to confirm the parasitic infection macroscopically and specimens were obtained for pathological studies.

Pathology: The resection material was fixed in 10% formalin, routinely processed and embedded in paraffin. Routine 5µ sections were stained with Haematoxylin and Eosin (H&E) for evaluation under light microscope. Additionally the sections were stained with Periodic Acid Schiff (PAS) stain in order to reveal laminated membranes.

RESULTS

The sonographic examinations of the infected animals revealed lobulated, heterogeneous cystic intraabdominal masses. There were echogenic solid areas with hypoechoic and anechoic cystic areas within the lesions. In most of the infected animals, the lesions were nearly occupying the entire intraabdominal cavity (Fig. 1a). The average size of the lesions were (2.2 ± 0.6) x (1.6 ± 0.5) x (2.8 ± 0.5) cm. There were no sign of solid abdominal organ infiltration or invasion on sonographic examinations of the infected animals as confirmed by the pathological investigation.

Doppler US examination revealed vascular nature of the heterogeneous cystic lesions. Color mode showed multiple vascular coding areas within the solid part of the lesions. Most of the vascular structures were venous with monophasic flow but there were also arterial vessels showing dominant flow with low resistive indices (0, 45 - 0, 55) (Fig. 1b).

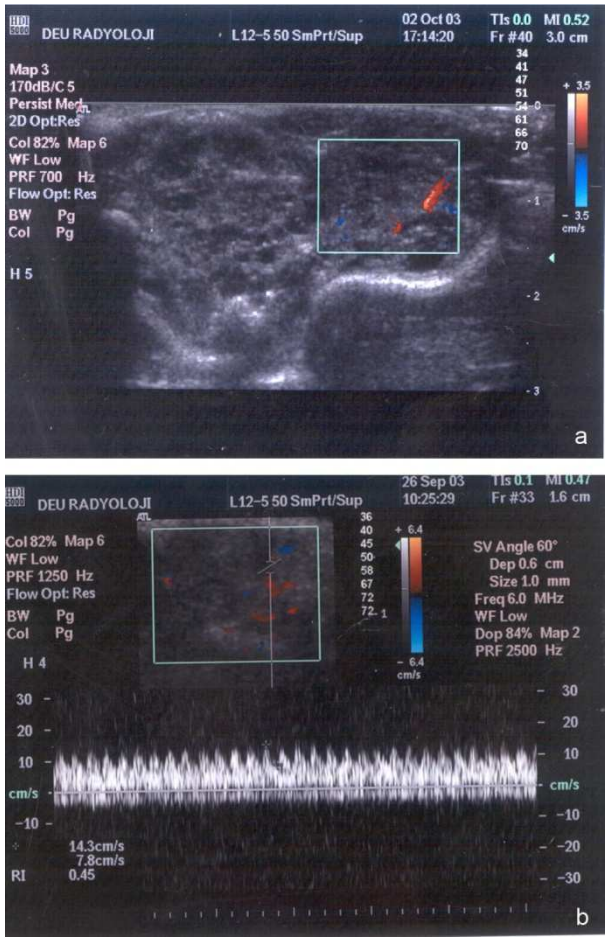

Figure 1. A. Longitudinal abdominal Doppler US of a *meriones ungiuculatus* infected with peritoneal implantation of Em, showing the vascular heterogenous mass lesion occupying the entire abdominal cavity with vascular color-coding areas. **B:** Spectral analysis of some of the vascular structures showing dominant arterial flow with low peripheral resistance (RI:0.45).

In the visual evaluation of the obtained specimens, it was noticed that there were considerably increased mesenteric vascularization in the abdomens of the infected animals (Fig. 2).

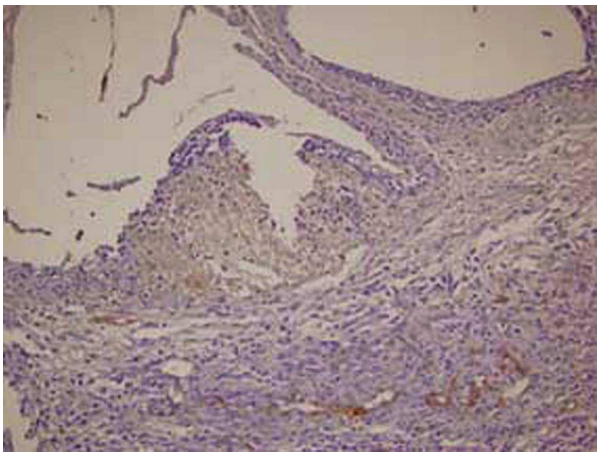

Figure 2. Histopathological examination revealed neovaskularization next to the cyclic structures within the granulation tissue (immune peroxidase staining, smooth muscle actin)

Haematoxylin and Eosin stained paraffin sections from gray white irregular resected tissue revealed multiple cystic spaces including PAS positive laminated membranes with mixed inflammatory infiltrate and granulation tissue formation including vascular structures around them. The vessels were mainly thin walled capillaries and there was no evidence of large mesenteric vascular invasion.

DISCUSSION

Diagnosis of Em infection can be established by imaging techniques, immunologic tests, or biopsy. Radiological patterns of Em lesions are often unreliable and difficult to interpret. Visualized lesions can be quite similar to those seen in primary hepatic tumors and metastatic neoplasms. Diagnosis is possible when the imaging findings are correlated with appropriate clinical and serological findings (1, 3, 8). Computed tomography, magnetic resonance imaging, Ultrasonography of Alveolar echinococcosis appears to be the most useful methods of diagnostic imaging (2). Radiological evaluation of the disease may be misdiagnosed as carcinoma of the liver since it yields a mass like appearance (1). We have demonstrated that metacestodes in tissue pieces that were obtained from a patient with alveolar echinococcosis have successfully been developed in *M. unguiculatus* and *Rattus norvegicus*, and this was the very first report of Em strain isolation in Turkey and nuclear diagnoses method (4-6)

Thus, further techniques such as Doppler US may be needed for differential diagnose for vascularization. A recent clinical study revealed the value of contrast enhanced Doppler US imaging findings for the evaluation of hepatic Em lesions (13). They showed that the hypovascularity of the inflammatory lesion might help the imaging and diagnosis of Em lesions. However in our experimental study the interesting finding was increased mesenteric vascularization with low resistance flow patterns within the non-visceral mesenteric Em implants. These vascular structures were supposed to be the invaded native mesenteric vessels within the infiltrating inflammatory mass lesions although in paraffin sections these vessels were seemed to be a part of granulation tissue formation around the laminated membranes. Doppler US finding of low peripheral resistance is interesting for the evaluation of this experimental Em model. We can not clearly differentiate the irregular borders of the lesions within the mesentery but the finding of low resistance is probably related to the infiltration of mesenteric structures with these inflammatory Em lesions and the arteries are feeding both the normal intestinal-omental structures and the granulomatous mass lesions.

The findings of our experimental study may help in the follow-up of patients with Em infection especially after abdominal operations and in the evaluation of Em lesions involving organs other than the liver.

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