Significance of Imaging Features of Alveolar Echinococcosis in Studies on Nonhuman Primates

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Abstract. In this study, we report the imaging findings in two Japanese monkeys (Macaca fuscata) diagnosed with alveolar echinococcosis. Both monkeys were treated with albendazole for 10 years, without surgery. Radiography, computed tomography, and contrast-enhanced ultrasonography were performed under general anesthesia. This is the first report on contrast-enhanced ultrasonographic imaging for alveolar echinococcosis wherein perfluorobutane was used as the contrast medium. The findings of the imaging analyses were similar to those reported for alveolar echinococcosis in humans, such as snowflake sign and worm-eaten sign. In addition, the serology correlated well with the imaging data in these two monkeys. Therefore, we propose that the imaging findings of alveolar echinococcosis in nonhuman primates may be used to accumulate data on this condition in human alveolar echinococcosis.

INTRODUCTION

Alveolar echinococcosis (AE) is a fatal zoonosis caused by the metacestode of Echinococcus multilocularis, an endemic pathogen found in the northern hemisphere, including the central part of Western Europe, and parts of the Near East, Russia, China, United States (Alaska), and Japan (Hokkaido). Foxes and dogs are the definitive hosts, whereas humans constitute one of the intermediate hosts along with nonhuman primates and rodents. After ingestion of the embryonated eggs by intermediate hosts, the embryos penetrate the intestinal mucosa, enter the portal circulation, and are typically carried to the liver. The incidence of AE is relatively lower than cystic echinococcosis caused by Echinococcus granulosus; however, it is a fatal disease with a mortality rate of over 90% in untreated or in inadequately treated patients within 10–15 years of diagnosis. 1–5

Alveolar echinococcosis most commonly affects the liver but mimics a malignant tumor by invading the adjacent tissues. 9 This unique feature of AE interferes with its primary diagnosis. As a result, early detection of AE is difficult even with computed tomography (CT) or ultrasound, and in many cases, the definitive diagnosis is made serologically after the lesions have progressed. One of the reasons for this difficulty is the lack of an image database of AE showcasing the atypical imaging features, which is in turn attributable to low disease incidence, limited case reports of AE, and low recognition in the non-epidemic regions. Therefore, along with the accumulation of human AE image data, accumulation of data on nonhuman primates will significantly contribute to the accurate diagnosis of AE, provided the latter have features similar to the human data. There are some reports on the pathologic findings of AE in nonhuman primates 6–13; however, there are few reports on the imaging features in live nonhuman primates. Recently, we had an opportunity to examine Japanese monkeys (Macaca fuscata) infected with E. multilocularis, and their radiography, contrast CT, contrast ultrasound, and serologic data and the outcome of albendazole therapy were obtained. On the basis of these results, the similarity of the image data between humans and M. fuscata and the significance of the AE image features obtained from the nonhuman primates were discussed.

MATERIALS AND METHODS

This study involved two female M. fuscata monkeys (designated as monkeys 1 and 2, weighing 12.6 and 9.9 kg, respectively) — the last survivors of a previous outbreak of AE in a zoo in 1998. 12 During this outbreak, 12 monkeys were infected, and 10 of these monkeys died by 2008. Western blotting performed on biologic samples revealed that all of the infected monkeys tested positive for E. multilocularis. 12 The 12 infected monkeys had been housed in a single cage with a common roof. The infection route was suspected to be through willows that were obtained from the local woods and were used to enrich the cage environment. Echinococcus multilocularis infection was histopathologically confirmed in the 10 monkeys that did not survive. Monkeys 1 and 2, living in the same cage, are still alive; they have been treated with albendazole (Eskazole; GlaxoSmithKline, Tokyo, Japan) for 10 years (from January 1998 to March 2008, 10.0 mg/kg, twice a day, administered in 4-week cycles at 14-day intervals). 5

Ten years ago, both monkeys were found to have a typical cystic lesion (measuring 52 × 51 mm in Monkey 1 and 93 × 75 mm in Monkey 2) in the right hepatic lobe; however, surgery was not performed. All examinations in this study were performed under general anesthesia maintained by isoflurane. Computed tomography images of the whole body were acquired using multidetector-row CT (Asteion Super 4; Toshiba, Tokyo, Japan). Iohexol (600 mgI/kg) (Omnipaque 300; Daiichi-Sankyo, Tokyo, Japan), a non-ionic iodinated contrast medium, was used for the contrast study. Ultrasonographic examination (Xario, Toshiba) of the abdomen and contrast study with 0.015 mL/kg perfluorobutane (Sonazoid, Daiichi-Sankyo) were performed. Si multaneous, serum samples were subjected to Western blotting. 14 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a crude E. multilocularis
antigen preparation that was formulated, as described by Laemmli. The antigens were electrophoretically separated using polyacrylamide gels containing 15% SDS (for 18 kDa antigens) and 8% SDS (for 55- and 66-kDa antigens), and the separated antigens were electroblotted onto a nitrocellulose membrane. The membrane was subsequently blocked in 5% skimmed milk in phosphate-buffered saline (PBS) for 1 hr at room temperature (RT) and then incubated with monkey sera (1:100 in PBS containing 0.05% Tween 20 and 5% skimmed milk) for 1.5 hr at RT. The membrane was washed and treated for 1 hr at RT with an alkaline phosphatase-conjugated anti-monkey IgG antibody (A-1929; Sigma, St. Louis, MO) diluted at 1:2,500 in 0.05% Tween-PBS. After further washing, the membrane was exposed to an alkaline phosphatase substrate solution (5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium; PerkinElmer Life Sciences, Gaithersburg, MD) for color development. In addition, enzyme-linked immunosorbent assay (ELISA) was performed, as described previously by Yamano and others.

**RESULTS**

The results of Western blotting revealed that Monkey 1 tested negative for AE, whereas Monkey 2 tested positive. Likewise, the ELISA value of Monkey 1 became negative after previously being positive, whereas that of Monkey 2 remained in the positive region (Table 1). The previous cystic lesion in Monkey 1 was completely calcified, and its diameter had reduced to 5.0 × 6.0 mm. On the other hand, although the cyst in Monkey 2 had decreased in size (30.5 × 25.0 mm) and the outer wall was calcified, many new cystic and calcified lesions were noted in the liver, biliary tract, uterus, and abdominal cavity (Figure 1). Furthermore, lesions with central liquefactive necrosis that were surrounded by a calcified cyst wall were also observed. A CT image (Figure 2A) revealed the largest cyst in the abdomen with a detached, delaminated layer, and a calcified outer wall, which is reported to be a finding of degenerating cysts. In addition, ultrasonographic images (Figure 2B) showed the snowflake sign, which is reported to be a finding of floating protoscoleces. Additionally, in the hepatic parenchyma of Monkey 2, a tumor-like, non-calcified, infiltrative lesion without a well-defined border was detected by CT and ultrasonography. It was difficult to diagnose this lesion without performing contrast CT and ultrasonographic examinations (Figure 3). Ultrasonographic images of the liver showed the “worm-eaten sign,” which is a characteristic finding of AE. In addition, CT and ultrasonography revealed that the spleen was absent in Monkey 2, although this monkey had not undergone splenectomy.

**DISCUSSION**

The present imaging examinations of the two monkeys revealed many features characteristic of AE, such as metastasis to various organs, calcified lesions, and reduction in lesion...
size after albendazole administration. Some of the other findings observed were detached germinal layer inside a cyst, which indicates cyst degeneration, and the worm-eaten sign, which is a finding exclusively observed in AE. These findings are similar to those reported in humans; therefore, it was suggested that the imaging features of AE in nonhuman primates may be used to accumulate imaging data on human AE. In addition, we obtained CT and ultrasonographic data of an infiltrative hepatic lesion without a well-defined border, which is occasionally difficult to distinguish from human hepatic tumors. This is the first report of contrast-enhanced ultrasonographic imaging of AE with perflubutane as the contrast medium. In addition to the progress in imaging diagnosis, there have also been advances in serologic diagnosis. In the current study, the results of Western blotting, ELISA, and imaging diagnosis agreed well. Thus, it is important to perform imaging diagnosis and serologic diagnosis simultaneously.

The cystic lesion in Monkey 1, which had been detected 10 years ago, was completely calcified and had decreased in size. In most situations, completely calcified lesions of AE correspond to inactive lesions. The reason for the difference in the treatment outcomes between monkeys 1 and 2 despite the identical treatment protocol followed using albendazole remains unknown. There are some reports that describe the acceleration in AE progression when the patients have immunologic abnormalities such as hepatitis B virus infection and human immunodeficiency virus infection. It is hypothesized that the absence of a spleen in Monkey 2 may have played a role in the low therapeutic effect and lesion progression. Attention needs to be paid to cystic lesions with or without calcification in patients with asplenia. It was impossible to determine whether albendazole or spontaneous death of the parasite contributed to AE termination in Monkey 1; however, it is interesting that the outcome of a successful parasitostatic treatment without surgical treatment could be monitored by imaging and serologic diagnoses.

The diagnosis of AE is difficult; however, it is relatively easy for doctors in endemic regions to list AE for differential diagnostic consideration.

**Figure 2.** Images of the largest cyst in the abdominal cavity of Monkey 2. (A) Transverse computed tomography (CT) image. The outer wall of the cyst is calcified. The germinal layer is detached from the outer wall (arrow). (B) Ultrasonographic image of the same cyst. The snowflake sign, which represents free-floating protoscoleces, is seen in the cyst.

**Figure 3.** Hepatic tumor-like lesions in Monkey 2. (A and B) Transverse computed tomography (CT) images, and (C and D) ultrasonographic images obtained before and after contrast administration, respectively. The arrows show active cysts in the liver. It is difficult to recognize the lesions in the non-contrast-enhanced images. The worm-eaten sign can be seen in Image D.
diagnosis. The problem is whether doctors in non-endemic regions can diagnose AE in their patients traveling or shifting to endemic regions. In most cases, the patient’s history might not be helpful in providing hints indicating AE because the incubation period of AE is usually long. In most cases, symptomatic treatments are performed over a long time period without definitive diagnosis, and AE gradually progresses. The diagnosis of AE in the early phase is quite important to increase the complete recovery rate with surgical treatment. Therefore, it is important to accumulate clinical data such as those from CT and ultrasound, which are non-invasive and performed at a relatively early stage of clinical course to arrive at a method to distinguish AE from confusing factors such as malignant tumors, pseudocysts, and pseudomyxomas. Moreover, the differential diagnosis of AE from other parasitic disease is also difficult; thus, a worldwide database should be created for the differential diagnosis of AE from other parasitic diseases such as fascioliasis, toxocariasis, trichinosis, strongyloidiasis, and cysticercosis. However, the human imaging features of AE have not been fully accumulated. Therefore, focusing on nonhuman primates for the accumulation of typical and atypical imaging patterns is of value in this regard. In addition, it is important to collect serologic data of nonhuman primates and imaging data because the findings of imaging and serology agreed well in this study. However, future studies are required to establish the validity of this data because there are possibilities that the pathologic findings and immunologic defense mechanisms of nonhuman primates can be different from those of humans, depending on the kinds of nonhuman primates.

Alveolar echinococcosis may spread to not only the liver but also the whole body, and it tends to present atypical clinical or imaging features in many cases; to add to this, the number of relevant case reports is less. Therefore, it is quite difficult to establish a diagnostic imaging database for differential diagnosis. However, this might be possible if the image information of AE can be shared between human and veterinary medicine because the latter has access to the image data of zoo animals and the captured wild animals. The survival rate of AE patients will increased considerably if the AE image data of nonhuman primates are accumulated and added to those of the humans, and more specific image findings or patterns than those currently reported will be discovered from the database.

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