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Analysis of contrast-enhanced ultrasound (CEUS) and pathological images of hepatic alveolar echinococcosis (HAE) lesions

H. ZHANG[1], Z.-H. LIU[2], H. ZHU[1], Y. HAN[2], J. LIU[1], L.-Q. DENG[1]

[1]Department of Ultrasonography, Sichuan Academy of Medical Science & Sichuan Provincial People's Hospital, School of Clinical Medicine, University of Electronic Science and Technology of China, Chengdu, China
[2]Department of Ultrasonography, People's Hospital of Aba Tibetan Qiang Autonomous Prefecture, Maerkang, China

Hui Zhang and Zhihong Liu contributed equally to this study

Abstract. – OBJECTIVE: Contrast-Enhanced Ultrasound (CEUS) has been introduced as a promising imaging technique for diagnosis of hepatic alveolar echinococcosis (HAE). But the correlation between the image features and the underlying complex pathology of HAE has not been fully understood. In this study, we reviewed CEUS and pathological images of 31 lesions in 24 patients with HAE from Aba Tibetan Qiang Autonomous Prefecture, an epidemic area in China.

MATERIALS AND METHODS: 24 patients who received CEUS examination for suspicion of HAE and with pathologically confirmed HAE were retrospectively reviewed. Parasitic lesions obtained from surgery were sectioned and stained with hematoxylin and eosin (HE).

RESULTS: US examination showed that the 12 lesions were hypoechoic and 19 lesions were hyperechoic. The hypoechoic images of HAE quite resemble the images of hepatocellular carcinoma and hemangioma. For the CEUS images of lesions ≥3 cm, 9 lesions (9/25, 36%) were hypoechoic with mixed content without circular rim enhancement; 16 lesions (16/25, 64%) had circular rim enhancement. All the lesions < 3 cm (n=6) were with circular rim enhancement and non-enhancement internal area. Pathological examination showed that the cysts are surrounded by an inner necrotic zone and peripheral granulomatous and fibrous tissues.

CONCLUSIONS: CEUS images of large HAE lesions are more complex than that of the small lesions. Large HAE lesions can be hypoechoic with mixed content with or without circular rim enhancement, and no internal area enhancement with circular rim enhancement. The small lesions are more likely to show circular rim enhancement and non-enhancement internal area.

Key Words:
Contrast-enhanced ultrasound, Liver, Alveolar Echinococcosis, *Echinococcosis multilocularis*.

Introduction

Hepatic Alveolar Echinococcosis (HAE) is a rare and chronic parasitic disease caused by larva of *Echinococcus multilocularis (E. multilocularis)*, which is characterized as a benign disease with infiltration of numerous small vesicle in tissue[1-3]. The primary lesions of *E. multilocularis* in the host usually occur in liver[4]. The pathological development of this disease quite resembles a slowly growing malignant tumor, such as gradual infiltrative growth, invasion of adjacent tissues, and distant metastasis[5]. This parasitic disease has a long latent stage before signs and symptoms show up[6]. Therefore, a large proportion of the patients is diagnosed with the late-stage infection. If untreated, the 10 years mortality reaches 75-100%[7-9].

Tibetan communities in Sichuan Province is one of the major endemic regions in China[10]. The unique lifestyle makes the herdsmen more susceptible to the parasites. One previous study reported that the prevalence of HAE is around 6% in this area[11]. Although radical surgery is the primary treatment for this disease, for the patients in advanced infection stage, radical surgery sometimes cannot be applied due to numerous violations in the liver[12,13]. Therefore, early diagnosis is quite necessary and helpful. In addition, even for the patients after radical surgery, imaging tech-

Corresponding Author: Liqiang Deng, MD; e-mail: 809196529@qq.com
Jun Liu, MD; e-mail: 1441228587@qq.com

niques are necessary for the follow-up of patients to assess the efficacy of the therapy and to monitor the viability of the parasite[14,15].

The conventional ultrasound (US) and the conventional radiological imaging techniques are not well adapted to the diagnostic purpose of this disease[15]. Contrast-Enhanced Ultrasound (CEUS) has been introduced as a promising imaging technique for diagnosis of HAE due to its low cost, easy manipulation and relatively high accuracy. Although the use of SonoVue as the contrast agent has significantly improved the demarcation of the lesion in CEUS, the correlation between image features and the underlying complex pathology of HAE has not been fully understood[15,16]. Only limited studies with small sample sizes assessed their association[15,17,18]. Therefore, in this study, we reviewed CEUS and pathological images of 31 lesions in 24 patients from Aba Tibetan Qiang Autonomous Prefecture, an epidemic areas in China.

Patients and Methods

Patient data

This work was approved by the Ethics Committee of the People's Hospital of Aba Tibetan Qiang Autonomous Prefecture. 24 patients (17 male and 7 female, with an average age of 41.8 years (range 31-62 years) who received CEUS examination for suspicion of HAE and with pathologically confirmed HAE from September 2014 to October 2015 in the hospital were retrospectively reviewed. All of the patients were residents of the Aba Tibetan Qiang Autonomous Prefecture, where is an endemic area for HAE. After confirmation, all the patients received radical surgery.

US and CEUS Examination

CEUS examination was performed by using MyLab 30 Color Doppler equipment (Esaote, Genoa, Italy), with a 1-8-MHz convex transducer. The US system was equipped with contrast tuned imaging (CnTI) (Esaote). The US and CEUS evaluation were performed by two independent and experienced sonographers (Hui Zhang and Yan Han). The conventional US was firstly performed to identify the position, shape, boundary and inner echogenicity of the lesions. Also, color Doppler flow imaging (CDFI) was performed to detect blood flow within and surrounding the lesions. Then, the CPS mode was selected and optimized by setting the instrument to the low mechanical index (MI=0.17-0.19). The gain was set high and focus was set to the maximum penetration depth. 2.4 mL echo-enhancing agent SonoVue (Bracco Spa, Milan, Italy) suspension was injected intravenously via antecubital vein as a bolus within a period of 3 s, followed by an injection of 5 mL physiological saline flush (0.9%). Since the injection, the images of the target lesion and surrounding liver parenchyma were recorded continuously for 6 min. According to previous studies, the arterial phase was defined as the 10-35 s after contrast agent injection, the portal venous phase refers to 50-120 s after the infection and the late phase is 130-300 s after the injection. The real-time and continuous liver perfusion image and the echo intensity change were recorded using a digital video recorder. The satisfactory images were selected and the diameters of the lesions were measured. The level of enhancement in the lesion areas are defined as hyper-enhancement, iso-enhancement, hypo-enhancement and non-enhancement compared to the surrounding normal liver parenchyma.

Hematoxylin and Eosin (HE) Staining of HAE Specimen

The parasitic lesions after resection were fixed in 15% formalin immediately after resection. Then the samples were embedded in paraffin wax and were further sectioned into 4 μm slices. Then tissue silences were stained with hematoxylin and eosin (HE).

Results

The Basic Characteristics of Patients Reviewed

The basic characteristics of patients reviewed were summarized in Table I. A total of 24 patients (17 male and 7 female) received US and CEUS examination were included. The patients were divided into two groups according to lesion diameters: 5 patients with 6 lesions <3 cm, while 19 patients with 25 lesions ≥3 cm. 17 patients had lesions in the right hepatic lobe, while 7 patients had lesions in left hepatic lobe. 18 patients had a single lesion, 5 patients had two lesions and 1 patient had three lesions. Therefore, there are 31 lesions in total reviewed. All the patients underwent surgery and the lesions were confirmed histologically.

The US and CEUS Characteristics

US examination showed that the 12 lesions were hypoechoic (38.7%, 12/31) (Figure 1A and C and Figure 2, *orange arrow*) while 19 lesions were

Table I. The basic characteristics of patients reviewed.

	Lesion diameter (<3 cm)	Lesion diameter (≥3 cm)
No.	5	19
Age (mean ± SD)	39.2±7.3	42.1±8.9
Gender		
M	3	14
F	2	5
Lesion location		
right hepatic lobe	4	13
left hepatic lobe	1	6
Lesion number		
1	4	14
2	1	4
3	0	1
Lesion diameter (mean ± SD)	1.7±0.6	5.2±1.5

hyperechoic (61.3%, 19/31) (Figure 1B, orange arrow). All of the lesions had irregular margin. 15 lesions had scattered foci of calcification (48.4%) (Figure 1B). 8 lesions showed a pseudocyst with central necrosis and surrounded by an irregular hyperechoic ring-like region (Figure 1C). The hypoechoic images of HAE quite resemble the images of hepatocellular carcinoma and hemangioma,

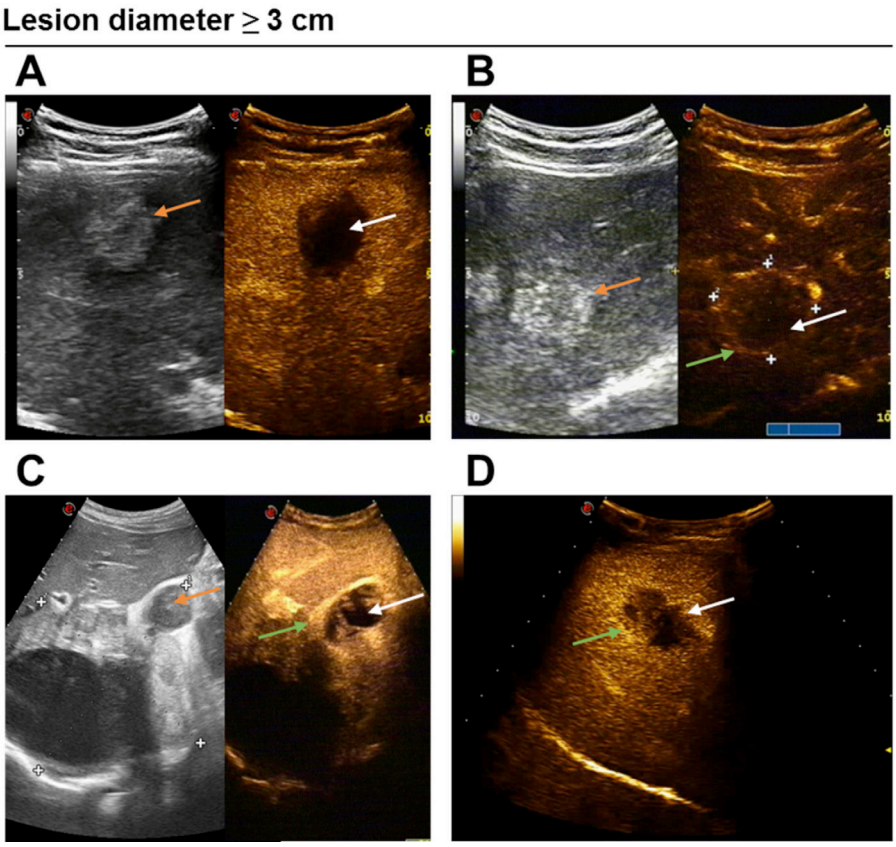

Figure 1. The US and CEUS images of HAE lesions larger than 3 cm. ***A-C,*** left: conventional US images; right: CEUS images; ***D,*** CEUS images. *Orange arrows*: Lesions examined by conventional US. *White arrows*: the non-enhancement and the hypo-enhancement internal area in the arterial phase by CEUS. *Green arrows*: the rim-like enhancement surrounding the lesions. Supplementary video (http://www.tudou.com/programs/view/IHOzc0yPXr8/). The US and CEUS recoding of the 23-year-old patient showed in Figure 1A.

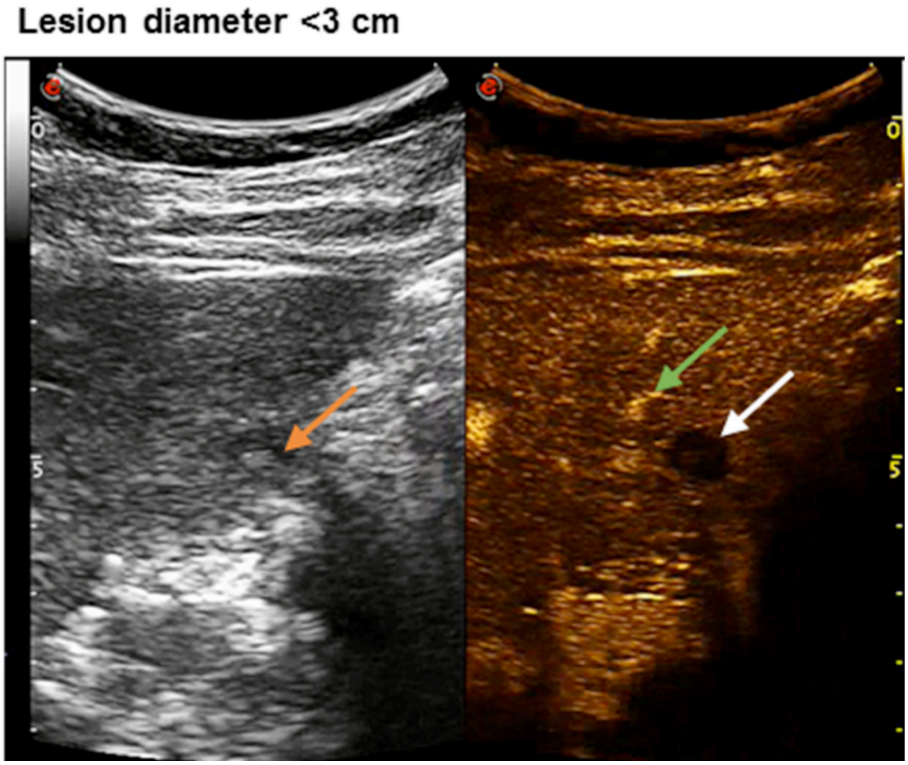

Figure 2. The US and CEUS images of HAE lesions smaller than 3 cm. *Orange arrow*: Lesion examined by conventional US. *White arrow*: the non-enhancement internal area in the arterial phase by CEUS. *Green arrow*: the rim-like enhancement surrounding the lesion.

which also presents hypoechoic substantial mass type. Therefore, this increases the possibility of misdiagnosis.

In the arterial phase, for the CEUS images of lesions 3 cm, 9 lesions (9/25, 36%) were hypoechoic with mixed content without circular rim enhancement (Figure 1A, *white arrow*); 16 lesions (16/25, 64%) had circular rim enhancement (Figure 1B-D, *green arrows*). Among the 16 lesions with rim-enhancement, 12 lesions were with nonhomogeneous hypo-enhancement (Figure 1B and D, *white arrows*) and 4 lesions were with no internal area enhancement (Figure 1C, *white arrow*). The US and CEUS recoding of the 23-year-old patient showed in Figure 1A was given in the Supplementary Video. For the CEUS images of lesions $\leq$ 3 cm, only one type of image was observed. All the lesions were with circular rim enhancement (Figure 2, *green arrow*) and non-enhancement internal area (Figure 2, *white arrow*), which resembles a small "black hole". In the portal-venous phase, contrast agent wash-out was observed in the hypo-enhancement area, while the non-enhancement area remained.

Pathological Results

Pathological sections of the lesions were stained with HE. In the lesion sections, there were multiple variable-sized multilocular cysts (Figure 3A). The cysts were surrounded by an inner coagulative necrotic zone (Figure 3A, short arrows) and peripheral granulomatous and fibrous tissues (Figure 3A, long arrows). In some large lesions, the necrosis was liquefactive (Figure 3B, short arrow, amplified in figure 3C). In the necrotic zone, peripheral inflammatory response area and remnant hepatic parenchyma could be observed (Figure 3B, long arrow, amplified in figure 3D). The peripheral inflammatory response area was barrier-like (Figure 3D, short arrow), which was quite distinctive to the remnant hepatic parenchyma (Figure 3D, long arrow).

Discussion

The accuracy of preoperative diagnosis of HAE is important for developing an appropriate therapeutic strategy. Since the introduction of

SonoVue as the contrast agent for CEUS, the diagnostic value of CEUS in HAE has significantly improved[15,16]. However, only limited studies with small sample size investigated the correlation between CEUS image features and the underlying complex pathology of HAE. Ehrhardt et al[18] compared with image features of lesions of HAE of CEUS, three-phase helical CT and FDG-PET. They observed that the images of CEUS were more consistent with FDG-PET in assessing the activity of HAE than CT. All positive FDG-PET findings were also positive on CEUS. Another study[16] reviewed 19 Chinese HAE patients with 19 lesions examined by the conventional US, CDFI and CEUS. They observed that 9 lesions showed irregular margin and hyperechoic mass and 10 lesions had mixed echogenicity type, with irregular hypoechoic mass in the central. CDFI confirmed that all of the lesions had no blood flow. CEUS imaging showed that all of the lesions had circular rim enhancement and a "black hole" (no enhancement in the central of lesion mass). Another recent study[17] investigated the features of small lesions (<3 cm) of HAE based on 9 patients with 17 lesions. Their data showed that the CEUS images of the lesions were all hypoechoic with mixed content. 12 lesions (70.1%) were rim enhanced with irregular piece-like non-enhanced internal areas during the arterial phase[17]. However, the number of patients and lesions reviewed in these studies were rather limited. It is recommended that the more CEUS images of HAE should be analyzed

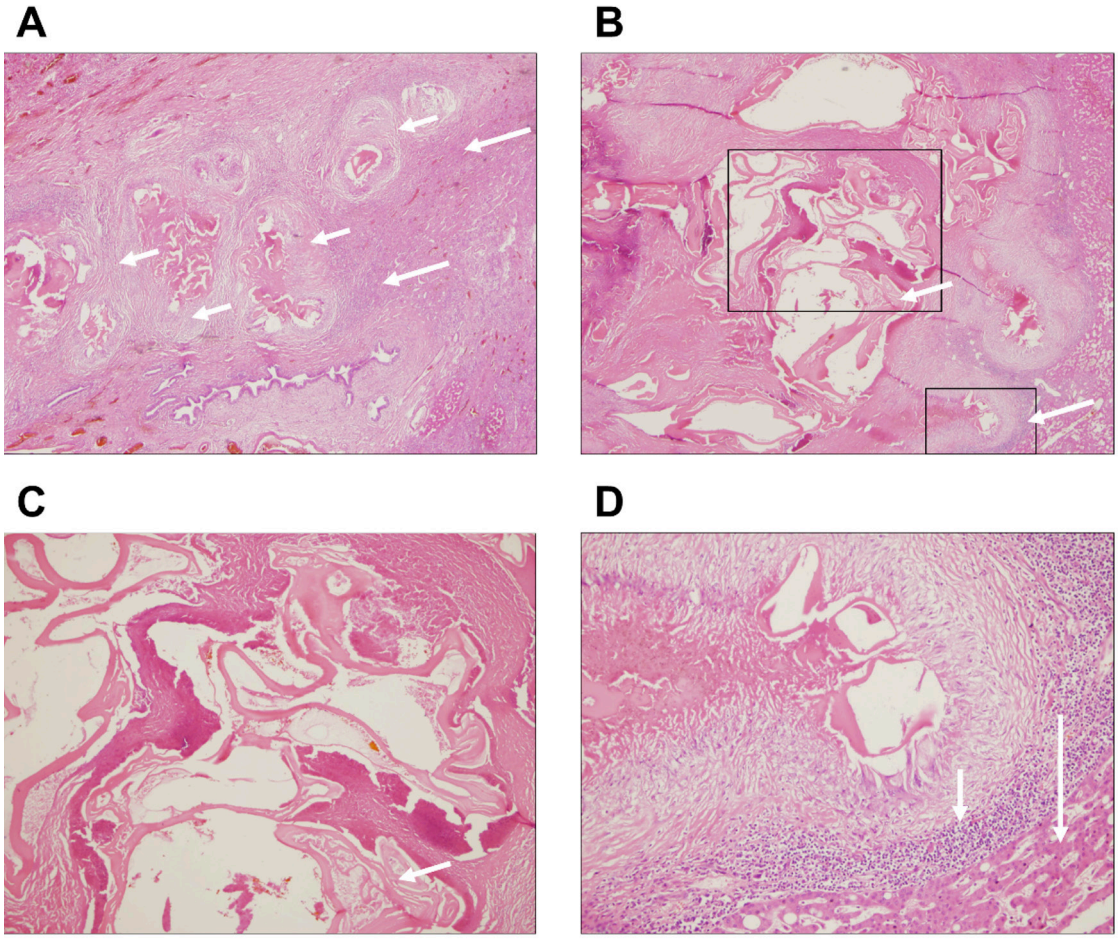

Figure 3. Pathological examination of the lesion sections. ***A,*** The parasitic cysts with surrounding necrotic area (*short arrows*) and peripheral granulomatous and fibrous tissues (*long arrows*) visualized microscopically with HE staining (40×magnification). ***B,*** A large parasitic cyst with liquefactive necrosis (*short arrow*, amplified in C) and peripheral inflammatory response area (long arrow, amplified in Figure D) (40×magnification). ***C,*** The 100×magnification of the indicating liquefactive necrotic area in figure B. ***D,*** The 200×magnification of the indicating area in Figure B. The peripheral inflammatory response area is barrier-like (*short arrow*), which is quite distinctive to the remnant hepatic parenchyma (*long arrow*).

before recommending CEUS as a standard imaging exam for HAE[15].

Another study[19] explored the pathological substratum of CEUS images based on a rat model. The report showed that the CEUS images of the lesions can be usually divided into two groups, including lesions with a diameter smaller than 3 mm or larger than 3 mm. The lesions smaller than 3 mm showed lesions showed rim enhancement in the peripheral area in the arterial phase and then showed no enhancement in either the portal or the delayed phase. In contrast, the lesions larger than 3 mm showed rim enhancement both in the arterial and portal phases. Pathological examination showed that parasitic vesicles had a surrounding inflammatory belt and formation of small peripheral vessels. For the lesions larger than 3 mm, pathological examination showed that the lesions were usually multiple vesicle-structures with surrounding inflammatory reaction belt and also the small peripheral vessels. SonoVue consists of phospholipid-stabilized microbubbles, which exerts contrast enhancing effects in blood vessels[16]. The small peripheral vessels around the lesions are no doubt the basis for enhancement. Therefore, CEUS can reflect the microcirculation of the lesions.

In the current study, we observed that CEUS characteristics of large HAE lesions (≥3 cm) are more complex than the small lesions (<3 cm). The images can be hypoechoic with mixed content with or without circular rim enhancement or with no internal area enhancement in combination with circular rim enhancement. As mentioned above, the rim enhancement is caused by enhancing effects in peripheral vessels around the lesions. However, the large lesions without the rim enhancement is likely due to the progression of the lesions and destruction of the peripheral vessels. As to the small lesions, the CEUS characteristics are quite consistent, all of the 6 lesions were with circular rim enhancement and non-enhancement internal area.

This work also has some limitations. Firstly, only five patients with small lesions (6 lesions in total) were reviewed. Therefore, the CEUS characteristics of small HAE lesions summarized in this study might not be representative. Secondly, the large lesions presented various features and some of them resembled the hepatocellular carcinoma and hemangioma. More investigations are required for better understanding of the unique features of HAE lesions.

Conclusions

CEUS image characteristics of large HAE lesions (≥ 3 cm) are more complex than that of the small lesions (< 3 cm). Usually, large HAE lesions can be hypoechoic with mixed content with or without circular rim enhancement and no internal area enhancement with circular rim enhancement. The small lesions are more likely to show circular rim enhancement and non-enhancement internal area.

Conflicts of interest

The authors declare no conflicts of interest.

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