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Original Article

Contrast-enhanced harmonic endoscopic ultrasonography for differential diagnosis of localized gallbladder lesions

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Background and Aim: Differential diagnosis of localized gallbladder lesions is challenging. The aim of the present study was to evaluate the utility of contrast-enhanced harmonic endoscopic ultrasonography (CH-EUS) for diagnosis of localized gallbladder lesions.

Methods: One hundred and twenty-five patients with localized gallbladder lesions were evaluated by CH-EUS between March 2007 and February 2014. This was a single-center retrospective study.Utilities of fundamental B-mode EUS (FB-EUS) and CH-EUS in the differentiation of gallbladder lesions and sludge plug were initially compared. Thereafter, these two examinations were compared with respect to their accuracy in the diagnosis of malignant lesions. Five reviewers blinded to the clinicopathological results evaluated microcirculation patterns in the vascular and perfusion images.

Results: In the differentiation between gallbladder lesions and sludge plug, FB-EUS had a sensitivity, specificity, and accuracy of 82%, 100%, and 95%, respectively, whereas CH-EUS had a sensitivity, specificity, and accuracy of 100%, 99%, and 99%, respectively. FB-EUS-based diagnosis of carcinomas based on tumor size and/or shape had a sensitivity, specificity, and accuracy of 61–87%, 71–88%, and 74–86%, respectively. Additional information regarding irregular vessel patterns in the vascular image and/or heterogeneous enhancement in the perfusion image on CH-EUS increased the sensitivity, specificity, and accuracy for the diagnosis of carcinomas to 90%, 98%, and 96%, respectively. There was a significant difference between FB-EUS and CH-EUS in terms of carcinoma diagnosis.

Conclusion: CH-EUS was useful for the evaluation of localized gallbladder lesions.

Key words: adenomyomatosis, contrast-enhanced harmonic endoscopic ultrasonography, endoscopic ultrasonography, gallbladder, gallbladder carcinoma

INTRODUCTION

ACCURACY OF DIFFERENTIAL diagnosis of gallbladder diseases by radiological imaging has recently improved, especially in the field of ultrasonography (US), where it has sometimes been difficult to differentiate benign disease from gallbladder carcinoma.[1–3] The majority of gallbladder carcinomas have a typical appearance on gray-scale sonography, with either a solid mass that occupies the whole gallbladder, or a focal polypoid mass.[4] A previous report suggested that all patients with gallbladder polyps larger than 10 mm in diameter should undergo resection.[5] This size criteria is insufficient to distinguish non-neoplastic from neoplastic polyps.[6] It has been proposed that color Doppler sonography and contrast-enhanced power Doppler US are useful techniques for the differential diagnosis of malignant and benign gallbladder disease.[7–11] However, such vascular imaging techniques carry a number of inherent limitations, including blooming or overpainting artifacts.[12–22] Recently, contrast-enhanced harmonic US (CE-US), has been recognized as a useful method for the diagnosis of gallbladder disease.[23–26] Additionally, endoscopic ultrasonography (EUS) is considered to be superior to US for depiction of the gallbladder, and provides high-resolution images.[27–29] As a combination of these techniques, known as contrast-enhanced harmonic EUS (CH-EUS), should be a powerful diagnostic approach,[30–32]

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we used CH-EUS for the differential diagnosis of gallbladder diseases, and evaluated its utility for their characterization.

METHODS

Patients

BETWEEN MARCH 2007 and February 2014, patients suspected of having localized gallbladder lesions according to conventional EUS at Kindai University Hospital underwent CH-EUS. This retrospective cohort study included retrospective review of imaging, clinical, and pathological data, with additional independent review of the CH-EUS videos. This study was carried out with the approval of the ethics committee of the Kindai University Faculty of Medicine.

Endoscopic ultrasonography

A GF-UCT260 echoendoscope (Olympus Medical Systems Co. Ltd, Tokyo, Japan) specifically developed for CH-EUS was used. EUS images were analyzed using an ALOKA ProSound SSD α-10 system (ALOKA Co. Ltd, Tokyo, Japan). If lesions were detected on fundamental B-mode EUS (FB-EUS) images of the gallbladder (an echogenic structure, polypoid lesion, wall thickening, or heterogeneous region), images of the ideal scanning plane were displayed to portray the whole extent of the lesion. Thereafter, the imaging mode was changed to extended pure harmonic detection (ExPHD), which synthesized the filtered second-harmonic components with signals obtained from the phase shift for contrast-enhanced harmonic imaging. Transmitting frequency and mechanical index were 4.7 MHz and 0.3, respectively. Sonazoid (Daiichi-Sankyo, Tokyo, Japan), which consists of perfluorobutane microbubbles surrounded by a lipid membrane, was used as the US contrast agent for CH-EUS. A bolus injection of the US contrast agent (15 μL/kg bodyweight) was given. After US contrast infusion, vascular and enhancement patterns were assessed in real time by examination of continuous 0–15 s (vascular images) and 40–60 s (perfusion images) images, respectively.

Imaging analysis

FB-EUS

A localized gallbladder lesion was defined as a solitary gallbladder lesion (i.e. multiple lesions in the gallbladder were excluded for analysis regardless of invasion or metastasis to other organs). If the echogenic gallbladder structure moved in response to tapping on the right hypochondriac region of the body surface, or changed in shape when there was postural change from left lateral to supine position, the lesion was defined as biliary sludge. Any findings of gallbladder lesions, except for sludge plug, were classified into two categories: pedunculated lesions and sessile lesions. Sessile lesions were defined as those where the base of the lesion was wider than the protuberance of the lesion on FB-EUS.

CH-EUS

Vascular images were categorized according to three patterns: spotty vessels ('spotty vessels', flowing in the lesion), irregular vessels ('linear vessels', flowing from the periphery to the center of the lesion), or no vessels. Perfusion images were categorized into four patterns: homogeneous, homogeneous with clear perfusion defects, heterogeneous, and the absence of an enhancement pattern. These patterns in the vascular and perfusion images were modifications of the gallbladder lesion classifications for contrast-enhanced transabdominal US reported by Inoue *et al.*[23] All data were stored in a recording system and reviewed by five readers (S. Omoto, T. Miyata, K. Minaga, K. Yamao, and M. Takenaka) who were absent during the examination and unaware of the clinicopathological results. Interobserver variations in the CH-EUS vascular and enhancement patterns were assessed by calculating the κ-coefficient. When the independent conclusions of the five reviewers differed, the saved images were reviewed together, and re-evaluated until agreement was reached.

Final diagnosis of gallbladder lesions

Final diagnoses were made on the basis of surgical specimens. Inoperable cases of gallbladder carcinoma were diagnosed according to the histology or cytology of samples of metastatic lesions obtained by EUS-guided fine-needle aspiration (EUS-FNA). In other cases, the final diagnosis was confirmed by follow-up examinations for at least 24 months. If the lesions remained unchanged in appearance, they were diagnosed as benign. When lesions were not found in either the resected gallbladder, or in follow-up EUS examinations of the gallbladder, the observed abnormality was defined as biliary sludge.

Statistical analysis

First, the sensitivities and specificities of FB-EUS and CH-EUS for distinguishing between gallbladder lesions and sludge plug were evaluated. Second, the sensitivities and specificities of FB-EUS were evaluated for cases of gallbladder carcinoma defined according to a size of 1 cm or more (diagnosis by tumor size) and the presence of a

sessile lesion (diagnosis by tumor shape). The sensitivity, specificity, and accuracy of CH-EUS in the diagnosis of gallbladder carcinoma were calculated when the carcinoma was defined as the presence of irregular vessels or heterogeneous enhancement. McNemar's test was applied to evaluate differences between FB-EUS and CH-EUS in terms of the diagnosis of gallbladder lesions from sludge plug and the diagnosis of malignancy versus no malignancy. All analyses were carried out using the statistical software SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA). Differences were considered to be statistically significant when *P*-value <0.05.

RESULTS

ONE HUNDRED AND twenty-five consecutive patients were recruited to this study (Table 1). Of the 125 patients, 75 subsequently underwent surgery. For the remaining 50 patients, a final diagnosis was confirmed by follow-up examinations or by EUS-FNA. EUS-FNA confirmed the diagnosis in 15 patients with gallbladder carcinoma, whereas 35 patients with benign gallbladder lesions were diagnosed at a follow-up examination. These follow-up analyses or pathological examinations revealed that 31 patients had a carcinoma (Table 1). All gallbladder carcinomas were diagnosed histologically as adenocarcinoma. In all 31 cases, median size of the gallbladder carcinoma was 19.6 mm (range, 4–50 mm); median size in 16 resected cases was 12.3 mm (range, 4–24 mm). Union for International Cancer Control (UICC) classification (7th edition) of the 16 resected cases revealed that two cases were stage 0, 10 were stage I, three were stage II, and one case was stage IIIB. CH-EUS detected a polypoid lesion hidden within the biliary sludge in one case (Fig. 1). In this case, FB-EUS resulted in a diagnosis of sludge plug, without detection of the polypoid lesion within the biliary sludge.

Table 1 Characteristics of patients in the present study

Total no. patients	125
Mean age (years)	61 (19–82)
Sex, male : female	67:58
Acquisition of final diagnosis, *n* (surgically resected)	125 (75)
Sludge plug	29 (20)
Non-neoplastic polyp	31 (9)
Chronic cholecystitis	18 (14)
Adenomyomatosis	16 (16)
Carcinoma	31 (16)
UICC classification† (stage)	
0	2
I	10
II	3
IIIA	0
IIIB	1

†Resected cases only.
UICC, Union for International Cancer Control.

CH-EUS vascular and perfusion images analysis

Table 2 shows the results of CH-EUS vascular and perfusion images according to gallbladder lesion type. Reproducibility measures of interobserver assessments showed that the κ-coefficient for the three vascular and four perfusion images categories were 0.915 and 0.928,

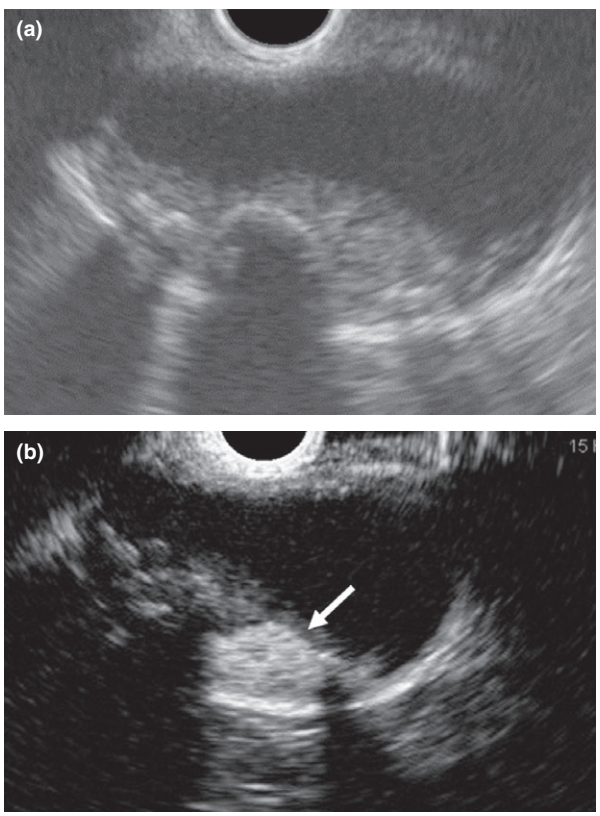

Figure 1 Case with a cholesterol polyp hidden by biliary sludge. (a) Fundamental B-mode endoscopic ultrasonography (monitor mode). A high echoic structure with acoustic shadow can be seen in the gallbladder. (b) Contrast-enhanced harmonic endoscopic ultrasonography (CH-EUS). The majority of the lesion is not enhanced by CH-EUS, but the polypoid lesion is incidentally detected in the center of the lesion as a homogeneous enhancement with spotty vessels (arrow).

Table 2 CH-EUS vascular and perfusion image diagnostic results for gallbladder lesions

	Vascular image (%)			Perfusion image (%)			
Gallbladder lesion (total, *n* = 125)	Spotty	Irregular	None	Homogeneous	Clear perfusion defects	Heterogeneous	Absent
Sludge plug (*n* = 29)	0	0	100	0	0	0	100
Non-neoplastic polyp (*n* = 31)	97	0	3	97	0	0	3
Chronic cholecystitis (*n* = 18)	100	0	0	100	0	0	0
Adenomyomatosis (*n* = 16)	87	13	0	0	87	13	0
Carcinoma (*n* = 31)	32	68	0	10	0	90	0

CH-EUS, contrast-enhanced harmonic endoscopic ultrasonography.

respectively. Vascular images showed that almost all gallbladder lesions, with the exception of the sludge plugs, contained spotty or irregular vessels, whereas no vessels were observed in any of the sludge plugs, or one of the 31 cases with polyps (3%). These lesions also showed no enhancement in the perfusion images (classified as 'absence of enhancement').

Classification of gallbladder lesions according to the combination of CH-EUS vascular and perfusion images

Gallbladder lesions were categorized into five types according to the combination of the CH-EUS vascular and perfusion images. The five categories and the classification results for the gallbladder pathology are shown in Figure 2. Type 1 lesions were characterized by no signs of blood flow (Fig. 3). Type 2 lesions were characterized by spotty vessels in the vascular image and a homogeneous enhancement pattern in the perfusion image (Fig. 4). Type 3 lesions were characterized by spotty vessels in the vascular image and a homogeneous pattern with clear perfusion defects in the perfusion image, suggestive of Rokitansky-Aschoff sinus (Fig. 5). Type 4 lesions were characterized by spotty vessels in the vascular image and heterogeneous enhancement in the perfusion image. Type 5 lesions were characterized by irregular vessels in the vascular image and heterogeneous enhancement in the perfusion image (Fig. 6).

Differentiation of solid lesions from sludge plug in FB-EUS and CH-EUS

For the differential diagnosis of gallbladder lesions from sludge plug, there was no significant difference between FB-EUS and CH-EUS although sensitivity and accuracy of CH-EUS were higher than those of FB-EUS (Table 3). Clinical characteristics of the five cases where biliary sludge in the gallbladder was misdiagnosed by FB-EUS, but correctly diagnosed by CH-EUS, are shown in Table 4.

Diagnosis of gallbladder carcinoma in FB-EUS and CH-EUS

Sensitivity, specificity, and accuracy of FB-EUS and CH-EUS in the diagnosis of gallbladder carcinoma are shown in

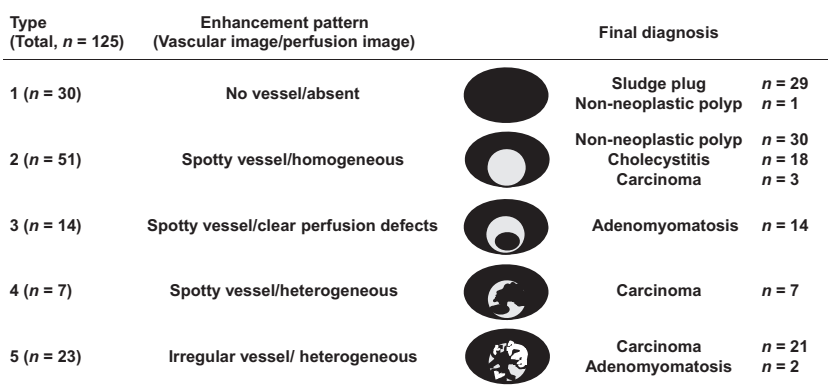

Figure 2 The five types of enhancement pattern and classification of gallbladder disease by contrast-enhanced harmonic endoscopic ultrasonography.

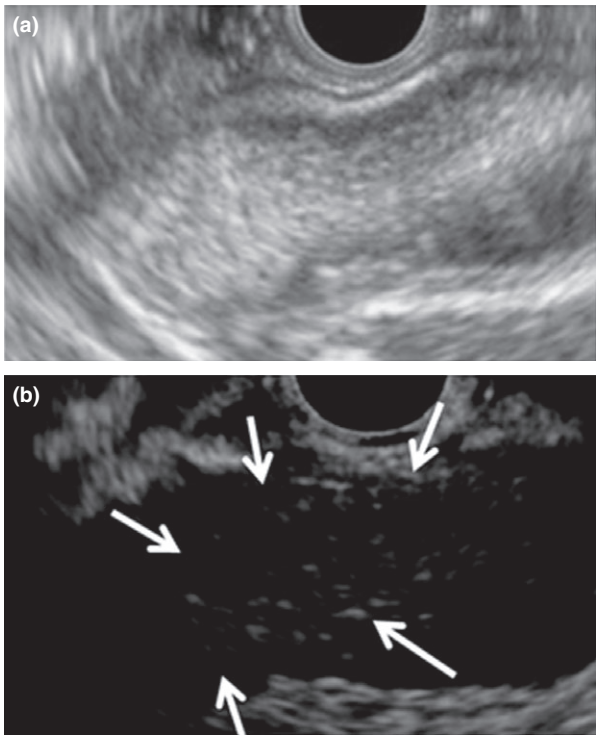

Figure 3 Typical case of gallbladder sludge plug. (a) Fundamental B-mode endoscopic ultrasonography (monitor mode). A high echoic structure can be seen in the gallbladder. (b) Contrast-enhanced harmonic endoscopic ultrasonography. The lesion is not enhanced (arrows).

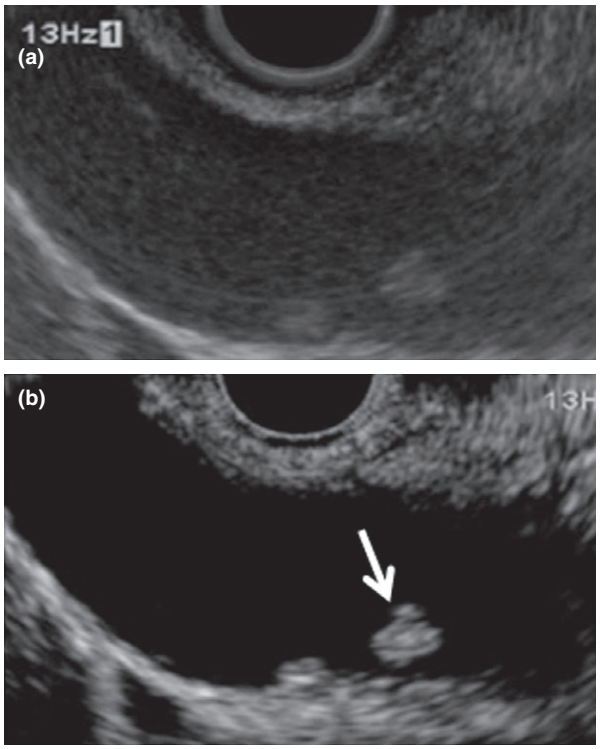

Figure 4 Typical case of a cholesterol polyp. (a) Fundamental B-mode endoscopic ultrasonography (monitor mode). An echogenic mass can be seen in the body of the gallbladder. (b) Contrast-enhanced harmonic endoscopic ultrasonography. The lesion (arrow) demonstrates homogeneous enhancement.

Table 5. When the tumor size classification was used for the diagnosis of gallbladder carcinoma, sensitivity, specificity, and accuracy were 77%, 88%, and 86%, respectively; thus, this diagnosis was more accurate than diagnosis based on tumor shape. Even when a diagnosis based on a combination of tumor size and shape was taken into consideration, the accuracy of FB-EUS did not improve.

Heterogeneous enhancement (equivalent to Type 4 and/or 5 diagnosed by CH-EUS) had a sensitivity, specificity, and accuracy for the diagnosis of the carcinoma of 90%, 98%, and 96%, respectively. There was a significant difference between a FB-EUS diagnosis based on tumor size, tumor shape, and their combination and a CH-EUS diagnosis based on heterogeneous enhancement ($P < 0.001$). Diagnosis of gallbladder carcinoma, based on interpretation of vascular and perfusion images by five independent observers, was also subject to receiver operating characteristic analyses. Area under the curve (AUC) was 0.880 for vascular images, 0.964 for perfusion images, and 0.966 for the combined images.

DISCUSSION

CONTRAST-ENHANCED HARMONIC US has been recognized as a useful tool for evaluation of the vascularity of gallbladder lesions.[23] Moreover, this technique is useful for both characterization of the lesion and observation of gallbladder wall integrity.[33] EUS enabled us to obtain more detailed images of the internal structure of gallbladder lesions;[34] therefore, in the present study we used CH-EUS using the ExPHD mode for the diagnosis and characterization of gallbladder lesions through evaluation of their vascularity and enhancement patterns. Doppler EUS with and without contrast enhancement allows the differentiation of gallbladder lesions from sludge plug by evaluation of their vascularity.[30] However, the Doppler mode cannot produce parenchymal perfusion images. When used with sonographic contrast agents, power or color Doppler EUS fails to depict signals from microbubbles in very slowly flowing microscopic vessels.[30,31] Power Doppler used with sonographic contrast agents is also prone to artifacts such as

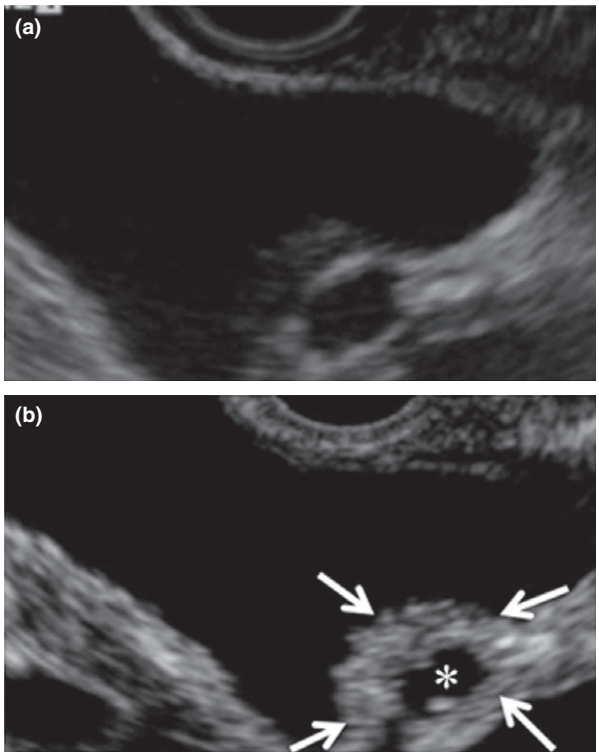

Figure 5 Typical case of adenomyomatosis. (a) Fundamental B-mode endoscopic ultrasonography (monitor mode). Localized wall thickening with an anechoic area is detected in the gallbladder. (b) Contrast-enhanced harmonic endoscopic ultrasonography. The lesion (arrows) is homogeneous, with a clear perfusion enhancement defect (*).

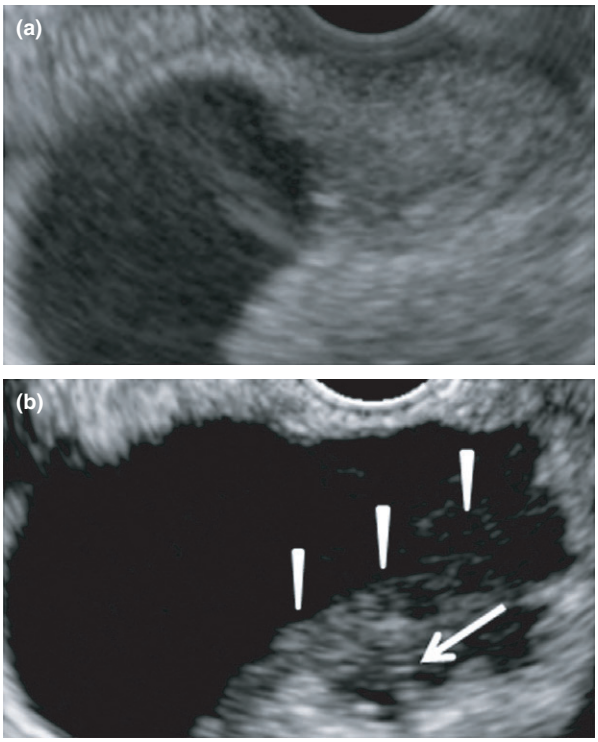

Figure 6 Typical case of gallbladder carcinoma. (a) Fundamental B-mode endoscopic ultrasonography (monitor mode). An echogenic structure is detected in the body of the gallbladder. (b) Contrast-enhanced harmonic endoscopic ultrasonography. The lesion (arrowheads) demonstrates irregular vessels with heterogeneous enhancement (arrow).

Table 3 Sensitivity, specificity, and accuracy of FB-EUS and CH-EUS for differentiating of solid lesions from sludge plugs

Examination	Sensitivity (%)	Specificity (%)	Accuracy (%)	*P*-value†
FB-EUS	82	100	95	0.221
CH-EUS (Type 1)‡	100	99	99	

†McNemar's test was used for comparing CH-EUS with FB-EUS.
‡No vessels and no enhancement observed on vascular and perfusion images.
CH-EUS, contrast-enhanced harmonic endoscopic ultrasonography; EUS, endoscopic ultrasonography; FB-EUS, fundamental B-mode EUS.

Table 4 Clinical characteristics of the five cases of biliary sludge in the gallbladder that were misdiagnosed as gallbladder lesions (except sludge plug) with FB-EUS

Patient no.	FB-EUS diagnosis	CH-EUS diagnosis†	Final diagnosis
1	Carcinoma	Type 1	Sludge
2	Carcinoma	Type 1	Sludge
3	Carcinoma	Type 1	Sludge
4	Benign lesion	Type 1	Sludge
5	Benign lesion	Type 1	Sludge

†Type 1, no vessel and absent enhancement observed on vascular and perfusion images.
CH-EUS, contrast-enhanced harmonic EUS; EUS, endoscopic ultrasonography; FB-EUS, fundamental B-mode EUS.

blooming, which makes the visualized blood vessels appear wider than in fundamental B-mode imaging.[30,31] By contrast, contrast harmonic imaging successfully provided images demonstrating parenchymal perfusion and microcirculation in gallbladder lesions.

There have been several reports on the differential diagnosis of gallbladder lesions, and these have described the use of a variety of imaging methods using contrast media. Xie *et al.*[33] reported that, in 90.9% of malignant

Table 5 Sensitivity, specificity, and accuracy of FB-EUS and CH-EUS for the diagnosis of gallbladder carcinoma

Examination	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)	*P*-value[†]
FB-EUS(1) Diameter >1 cm	77% (24/31)(0.64–0.87)	88% (83/94)(0.84–0.92)	86% (107/125)(0.70–0.90)	
(2) Sessile lesion	71% (22/31)(0.56–0.83)	79% (74/94)(0.74–0.83)	77% (96/125)(0.70–0.83)	
(3) (1) and/or (2)	87% (27/31)(0.73–0.95)	71% (67/94)(0.67–0.74)	75% (94/125)(0.68–0.80)	
(4) (1) and (2)	61% (19/31)(0.47–0.74)	79% (74/94)(0.74–0.83)	74% (93/125)(0.67–0.81)	
CH-EUS[‡](5) Type 4	23% (7/31)(0.18–0.26)	100% (94/94)(0.97–1.00)	81% (101/125)(0.78–0.82)	
(6) Type 5	68% (21/31) (0.53–0.69)	98% (92/94) (0.94–0.99)	90% (113/125) (0.84–0.92)	
(7) Type 4 and/or 5	90% (28/31) (0.81–0.95)	98% (92/94) (0.95–0.99)	96% (120/125) (0.91–0.98)	
(1) *vs* (7)				<0.001
(2) vs (7)				<0.001
(3) *vs* (7)				<0.001
(4) *vs* (7)				<0.001

[†]McNemar's test was used to compare CH-EUS with FB-EUS. For this analysis, (1) and (7), (2) and (7), (3) and (7), and (4) and (7) were compared.
[‡]Type 4, spotty vessel and heterogeneous enhancement observed on vascular and perfusion images; Type 5, irregular vessel and heterogeneous enhancement observed on vascular and perfusion images.
CH-EUS, contrast-enhanced harmonic endoscopic ultrasonography; EUS, endoscopic ultrasonography; FB-EUS, fundamental B-mode EUS.

lesions and in 17.0% of benign lesions, a pattern of hyper-enhancement or iso-enhancement was found in the early phase of contrast agent administration, which then faded out to hypo-enhancement within 35 s. They observed the washout of contrast from gallbladder lesions. One study reported on the utility of contrast-enhanced EUS for the diagnosis of gallbladder diseases; however, the authors used conventional EUS, which was not equipped with a specific mode for contrast enhancement.[35] According to their report, gallbladder carcinoma showed enhancement, whereas benign gallbladder polyps did not show enhancement. Intensity of contrast enhancement on conventional B-mode imaging was insufficient to permit visualization of the vascular structures. In our study, using Sonazoid and ExPHD mode, which emphasizes the microcirculation imaging effect due to the contrast agent, all gallbladder lesions showed vascularity, with the exception of 29 cases with sludge and one case with a non-neoplastic polyp (2 mm or less in diameter). Choi *et al.*[36] reported on the utility of CH-EUS using ExPHD mode. However, 14 of 59 benign polyps (24%) demonstrated no vascularity. This difference might be attributed to their use of SonoVue (Bracco, Milan, Italy) as a contrast agent.

On either transabdominal US or EUS, neoplastic gallbladder lesions sometimes exhibit similar features to other gallbladder lesions, such as cholecystitis, adenomyomatosis, and even biliary sludge.[1–3] We therefore evaluated the wall thickening of lesions in the gallbladder, as well as polypoid lesions. CE-US using coded phase inversion harmonic US with Levovist (Schering AG, Berlin, Germany) allowed more effective differential diagnosis between gallbladder sludge and gallbladder lesions (except for sludge plug) than conventional US.[23] In the present study, CH-EUS allowed differentiation of biliary sludge from neoplastic lesions according to the presence of vascularity. This EUS-based result was consistent with that of transabdominal US. CH-EUS demonstrated that adenomyomatosis was indicated by clear perfusion defects in the perfusion image, which may represent Rokitansky-Aschoff sinuses. Gallbladder carcinoma appears as an echogenic or echopenic mass without aggregation of echogenic spots, with the internal echo pattern of carcinoma often being heterogeneous.[37] Choi *et al.*[28] reported that an EUS-based scoring system aided the identification of malignant gallbladder polypoid lesions, and reported the sensitivity and specificity for differentiation of benign and malignant gallbladder polypoid lesions as 81% and 86%, respectively. In this study, we used a combination of tumor size and tumor shape for diagnosing gallbladder carcinoma, and similar results were observed for the diagnoses made on the basis of tumor size.

CH-EUS showed heterogeneous enhancement in 28 of 31 cases of carcinoma (Type 4 and/or 5). With the exception of two cases of adenomyomatosis, heterogeneous enhancement was not found in benign gallbladder lesions. These results reveal that perfusion image on CH-EUS has a high sensitivity and specificity for the diagnosis of gallbladder carcinoma. The combination of vascular and perfusion image improved the AUC in comparison with vascular image only. However, we cannot conclude that combining images improved the AUC over that obtained for perfusion images only; this is because, when the level of interobserver agreement about vascular and perfusion images is taken into account, the perfusion images alone appear to yield a more definitive diagnosis than combined images.

The present study has several limitations. Study weaknesses include the retrospective nature of the study and the lack of a control group. Endosonographers were not blinded to the sonography techniques; therefore, there may have been bias when carrying out EUS, such as focusing on certain characteristics according to the pretest probability of malignancy. Verification bias cannot be completely excluded, as three cases were affected by the CH-EUS results in terms of surgery.

In conclusion, CH-EUS was useful for depicting microcirculation, and for differential diagnosis of biliary sludge from other gallbladder lesions. Gallbladder carcinoma was characterized by irregular vessels in the vascular image, and heterogeneous enhancement in the perfusion image. Although this study examined a limited number of patients, and further studies with larger patient numbers are needed to confirm these conclusions, it can be concluded that CH-EUS provided significant improvements over conventional EUS with respect to the quality of diagnoses.

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CONFLICTS OF INTEREST

AUTHORS DECLARE NO conflicts of interest for this article.

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