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ORIGINAL ARTICLE–GASTROENTEROLOGY

Feasibility of endoscopic ultrasonography using a 60-MHz ultrasound miniature probe in the upper gastrointestinal tract

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Abstract

Purpose The use of higher frequencies in ultrasound allows for a more detailed image. This study aimed to investigate the feasibility of delineating the gastrointestinal wall using a 60-MHz miniature ultrasound probe.

Methods A phantom study was performed using a multipurpose ultrasonic phantom model, and the depth of imaging was evaluated using 60-MHz and 20-MHz miniature probes and 7.5-MHz conventional convex-type endoscopic ultrasonography. A total of 25 visualized areas from a total of 16 specimens from 16 patients were enrolled. The structures of the layers of the esophagus, stomach, and duodenum were evaluated using a 60-MHz probe and a pathological specimen created from endoscopically or surgically resected specimens.

Results The 60-MHz probe was able to render to a depth of 2 mm and visualize the esophagus, stomach, and duodenum in five layers, respectively, within the depiction range. The depiction ranges of the 20-MHz probe and 7.5-MHz conventional endoscopic ultrasonography were 5 mm and 60 mm, respectively. The 60-MHz probe visualized the muscularis mucosae as the fourth layer in the esophagus, the fourth layer in the stomach, and the second layer in the duodenum. Muscularis mucosae were delineated in almost all cases, except in two cases where the layered structure disappeared.

Conclusion The 60-MHz probe provided good visualization of the muscularis mucosae and structure of the layers down to the submucosa, which improves the ability to diagnose the depth of early cancer invasion of the upper gastrointestinal tract, leading to more appropriate treatments.

Keywords High-frequency ultrasound · High-frequency miniprobe · Invasion depth diagnosis · Muscularis mucosae · 60-MHz ultrasound probe

Introduction

Endoscopic treatment is considered the gold standard for shallow, invasive gastrointestinal cancer with a low risk of lymph node metastasis as it is minimally invasive and can also be curative [1–5]. To determine the indications for endoscopic treatment, it is crucial to accurately diagnose the invasion depth of the mucosa (M) or submucosa with infiltration less than 500 µm (SM1) in patients with gastric cancer and the muscularis mucosae (MM) or upper third of the submucosa (SM1) in patients with esophageal cancer. It is known that endoscopic ultrasonography enables observation of the layered structure of the gastrointestinal tract, and when used in combination with white light observation, the diagnosis of invasion depth is improved [6–8]. The visualization rate of the stomach MM is not sufficient with the 20-MHz miniature probe that is currently widely used [9].

The first endoscopic ultrasonography (EUS) instrument was an electronic linear scanning system developed by Green et al. of the Science Research Institute in 1980 [10, 11]. The diagnostic ability of EUS has recently improved with the development of contrast-enhanced EUS and EUS elastography [12, 13]. The miniature probe was first used

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in 1989 [14]. It is an indispensable modality for diagnosing gastrointestinal cancer depth as it can be used through the working channel of standard endoscopes, providing the ability to scan target lesions under direct visualization. With higher ultrasound frequencies, more detailed images can be obtained within the drawing range; however, as the frequency increases, the drawing depth becomes shallower. There are several reports of the use of high-frequency probes (up to 30 MHz), though no differences in the accuracies of the diagnosis of the depth of invasion have been reported between 20-MHz and 30-MHz probes [15–19].

Therefore, we created a prototype 60-MHz high-frequency probe in collaboration with Fujifilm Cooperation to improve the ability to diagnose gastric cancer in the M and SM1 and esophageal cancer in the MM and SM1, which are often difficult to diagnose. This study aimed to examine the ability of the 60-MHz probe to visualize the layers of the gastrointestinal wall.

Materials and methods

Phantom and specimen studies were performed to clarify the visualization ability of the 60-MHz miniature probe. The 60-MHz miniature probe is a prototype (Fujifilm Corp., Tokyo, Japan) with a 1-mm oscillator inside the sheath. The sheath was filled with saline for drawing (Fig. 1). The drawing was performed with the ultrasonic transducer positioned close to a submerged specimen as the probe is not sufficiently durable to use through the accessory port of an endoscope.

Phantom study

We used a multi-purpose ultrasonic phantom model made of low-damping rubber (Eastek Co., Tokyo, Japan) to evaluate the device's capabilities (Fig. 2a, b; Table 1). The phantom model contained ultrafine monofilaments with a diameter of 0.05 mm that were located at 1-mm intervals from the surface to a depth of 1–5 mm and at 5-mm intervals at a depth of 5 mm or more. We stored saline on the top of the phantom and visualized the model using the 60-MHz miniature probe, a 20-MHz probe (UM-3R; Olympus Co., Tokyo, Japan), and a 7.5-MHz conventional convex-type EUS (EG580UT, Fujifilm Corp.) (Table 2). We determined the maximum depth that could be drawn with each device as the depiction range.

Specimen study

This study was a single-center, prospective, observational study. The study included 16 patients who underwent endoscopic ($n=9$) or surgical ($n=7$) resection for

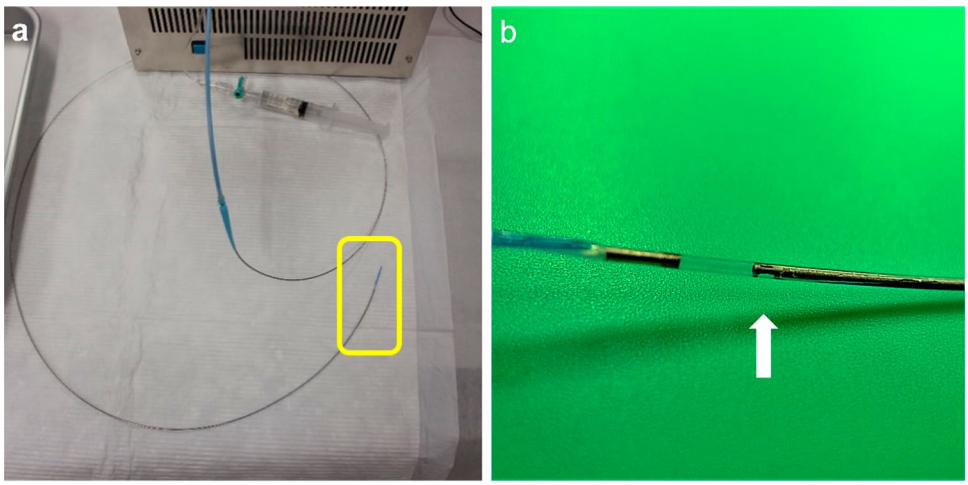

Fig. 1 The 60-MHz miniature probe is shown. The transducer is 1 mm (arrow)

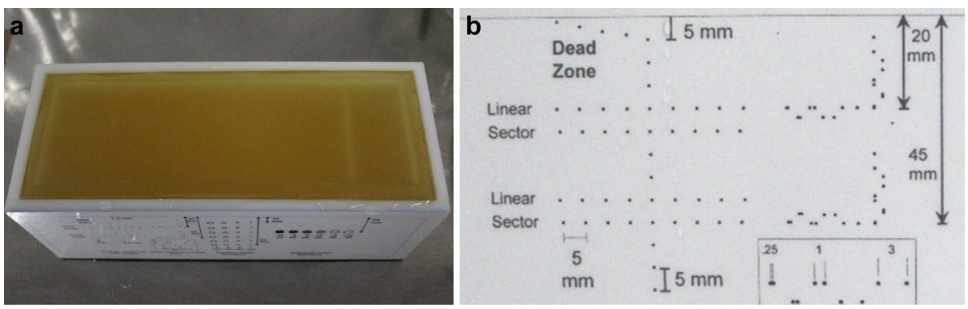

Fig. 2 The specifications of the multi-purpose ultrasound phantom model (Eastek Co., Tokyo, Japan) are shown. **a** The phantom model is made of low-damping rubber and contains ultrafine monofilaments. **b** The left-hand scale is used in this study

Table 1 Specifications of multi-purpose ultrasound phantom model*

Tissue mimicking material	
Type	Urethane rubber
Attenuation	0.5 dB/cm/MHz 5%
Speed of sound	1450 mps ± 1%@23℃
Line Target	
Material	Monofilament nylon
Diameter	0.05 mm ± 5.0%
Position tolerance	± 0.1 mm
Dead zone group	
Lateral displacement	5.0 mm
Interval spacing	1.0 m
Depth	1.0–4.0 mm

*Phantom model 551 (Eastek Co., Tokyo, Japan)

upper gastrointestinal tract cancer at our hospital between January 2015 and December 2016. The specimens were stretched and pinned onto a rubber plate with the luminal surfaces exposed immediately after excision.

The specimens were then submerged in saline and visualized using the probes. The visualized area was marked with a fixing pin. The resected specimens were fixed in 10% formalin after the study. After fixation, the visualized area was excised and stained with hematoxylin. The imaging and pathological results were then compared using cellSens (Olympus Co., Tokyo, Japan), which is an imaging software for microscopes that was used to measure the layer structure of the image and the pathological tissue.

The Institutional Review Board of Nagoya University Hospital approved this study (IRB approval number 2014-0224). This study is registered with UMIN (UMIN number UMIN000016279). Written informed consent was obtained from the patients.

Results

Phantom study

The 60-MHz probe depicted monofilaments within the phantom model up to a depth of 2 mm. The depiction ranges of the 20-MHz probe and 7.5-MHz conventional EUS were 5 mm and 60 mm, respectively (Fig. 3a-d).

Specimen study

The patient characteristics are shown in Table 3. Overall, a total of 25 visualized areas from a total of 16 specimens from 16 patients were included during the study period. The visualized areas included 11 tumors and four normal-tissue specimens from the esophagus, three tumors and three normal-tissue specimens from the stomach, and three tumors and one normal-tissue specimen from the duodenum.

The five-layered structure of the esophageal wall, gastric wall, and duodenal wall were delineated within the depiction range with the 60-MHz probe. The layers of the esophagus were arranged outwards as follows: a high echoic layer (first layer), thin low echoic layer (second layer), high echoic layer (third layer), low echoic layer (fourth layer), and high echoic layer (fifth layer). Similarly, the layers of the stomach were arranged outwards as follows: a high echoic layer (first layer), low echoic layer (second layer), high echoic layer (third layer), low echoic layer (fourth layer), and high echoic layer (fifth layer). The layers of the duodenum were arranged outwards as follows: a high echoic layer (first layer), low echoic layer (second layer), high echoic layer (third layer), low echoic layer (fourth layer), and high echoic layer (fifth layer).

CellSens measured each layer and structure of the ultrasonic image and the pathological specimen. In one case of

Table 2 Specifications of miniature probes and convex-type EUS

Type	Miniature probe		Convex type
Model	Prototype*	UM-3R**	EG580UT*
Display mode	B	B	B/M/D/CDFI/PDFI
Scanning method	Mechanical radial	Mechanical radial	Electronic curved linear array
Scanning direction	360°	360°	150°
Frequency (MHz)	60	20	7.5
Maximum diameter (mm)	1.0	2.5	15.0
Working length (mm)	1500	2050	1250
Range resolution	≦ 200 μm	≦ 1 mm	≦ 1 mm
Azimuth resolution	≦ 200 μm	≦ 2 mm	≦ 2 mm

B B-Mode, *M* M-Mode, *D* pulsed Doppler, *CDFI* color Doppler flow imaging, *PDFI* power Doppler flow imaging

*Fujifilm Co., Tokyo, Japan; **Olympus Co., Tokyo, Japan

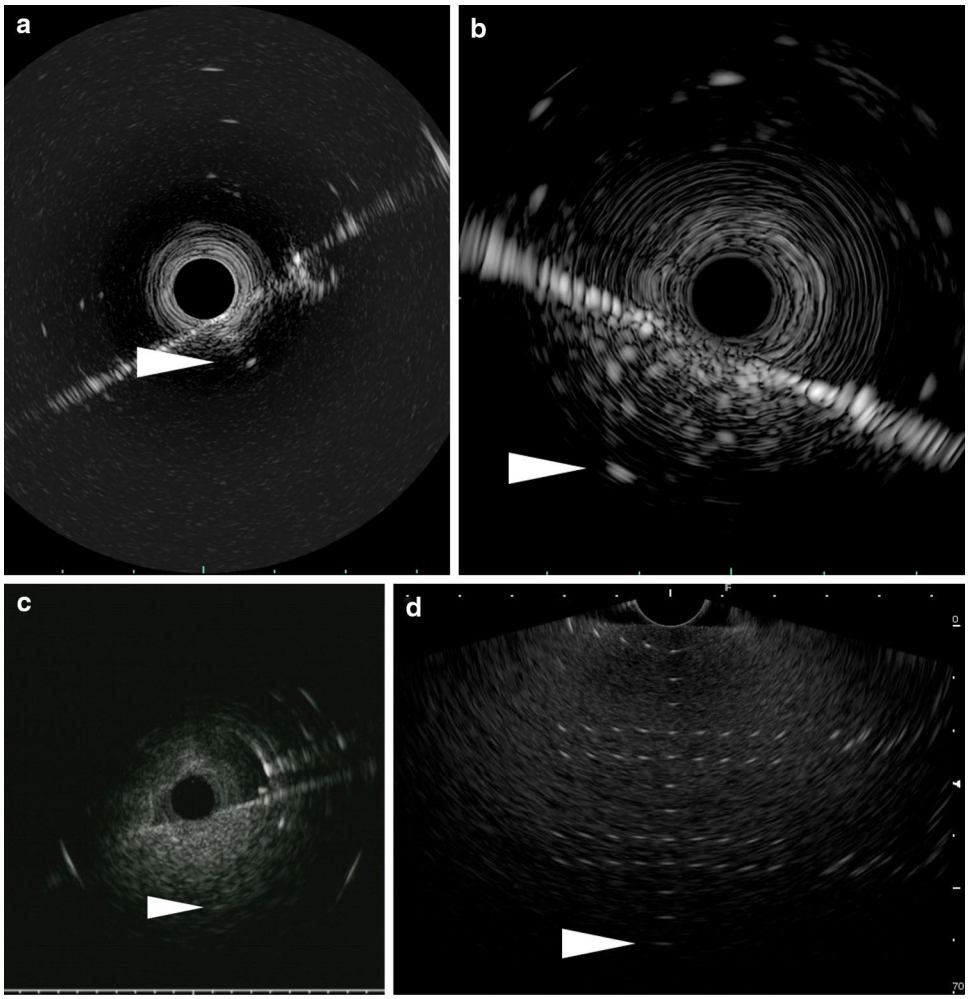

Fig. 3 Phantom study. The phantom is drawn with each probe and a conventional convex-type EUS. **a** 60-MHz miniature probe; 1 mm. **b** 60-MHz miniature probe; 2 mm. **c** 20-MHz miniature probe; 5 mm. **d** 7.5-MHz conventional EUS; 60 mm

normal esophagus, each layer's length on the ultrasound image was 376 µm in the first layer, 84 µm in the second layer, and 1031 µm in the first to fourth layers. The pathological specimens at the site estimated to correspond to them were 275 µm, 61 µm, and 766 µm, respectively (Fig. 4a, b). In one case of gastric adenoma, ultrasonographic images showed a vertical length of 606 µm in the anechoic region, which was presumed to be an expanded duct, and 1384 µm from the surface to the hypoechoic layer, which was presumed to be a muscularis mucosae. In contrast, it was 502 µm and 1113 µm on the pathological specimen, respectively (Fig. 4c, d). When measured by the same method for all specimens, the reduction rate of the pathological specimen after fixation was 73–80% compared to the ultrasound image, although there were differences depending on the site. The epithelium (EP) of the esophagus was visualized as a two-layer structure on the pathological specimen. The third layer was identified as the lamina propria mucosae (LPM), the fourth layer was the MM, and the fifth layer was the SM (Fig. 5a, b). In the esophagus, the median length from the mucosal surface to the center of the MM was 718 (450–1132) µm on EUS images and 548 (392–849) µm on pathological specimens, with a reduced rate of 76.3%.

In the stomach, the M was visualized as a three-layer structure and the fourth layer was identified as the MM and the fifth layer was the SM (Fig. 5c, d). The deep part of the SM of the stomach wall was not visualized when using the 60-MHz probe.

In the duodenum, M was visualized as the first layer, the second layer was the MM, the third layer was the SM, the fourth layer was the muscularis propria (MP), and the fifth layer was the serosa (S) (Fig. 5e, f). We confirmed a similar structure of the layers in all normal tissue specimens.

A tumor in the submucosa was depicted in one esophageal cancer specimen (Fig. 6a, b). The 60-MHz probe was able to depict tumor infiltration into the MM and differentiate MM and tumor (Fig. 6c, d). Dilated ducts were observed in specimens with gastric adenoma (Fig. 7a, b) and duodenal adenoma (Fig. 7c, d). The visualization rate of the MM of the upper gastrointestinal tract was 92% (23/25) using the 60-MHz miniature probe. In a case that had undergone chemoradiotherapy, the layer structure disappeared due to

Table 3 Patient characteristics

			$n=16$
Male: female			12:4
Age: median (range)			71 (57–78)
Therapy	Endoscopic resection	ESD	7
		EMR	2
	Surgical resection	Subtotal esophagectomy	5
		Pancreatic duodenectomy	1
		Local excision (duodenum)	1
Specimen	Normal tissue	Esophagus	4
		Stomach	3
		Duodenum	1
	Tumor	*Esophagus*	8
		Esophageal cancer	5
		LPM	1
		MM	1
		SM2	1
		AD	1
		Dysplasia	2
		Other	
		Stomach	2
		Gastric cancer	2
		M	1
		Gastric adenoma	
		Duodenum	2
		Duodenal cancer	2
		M	1
		Duodenal adenoma	

ESD Endoscopic submucosal dissection, *EMR* Endoscopic mucosal resection

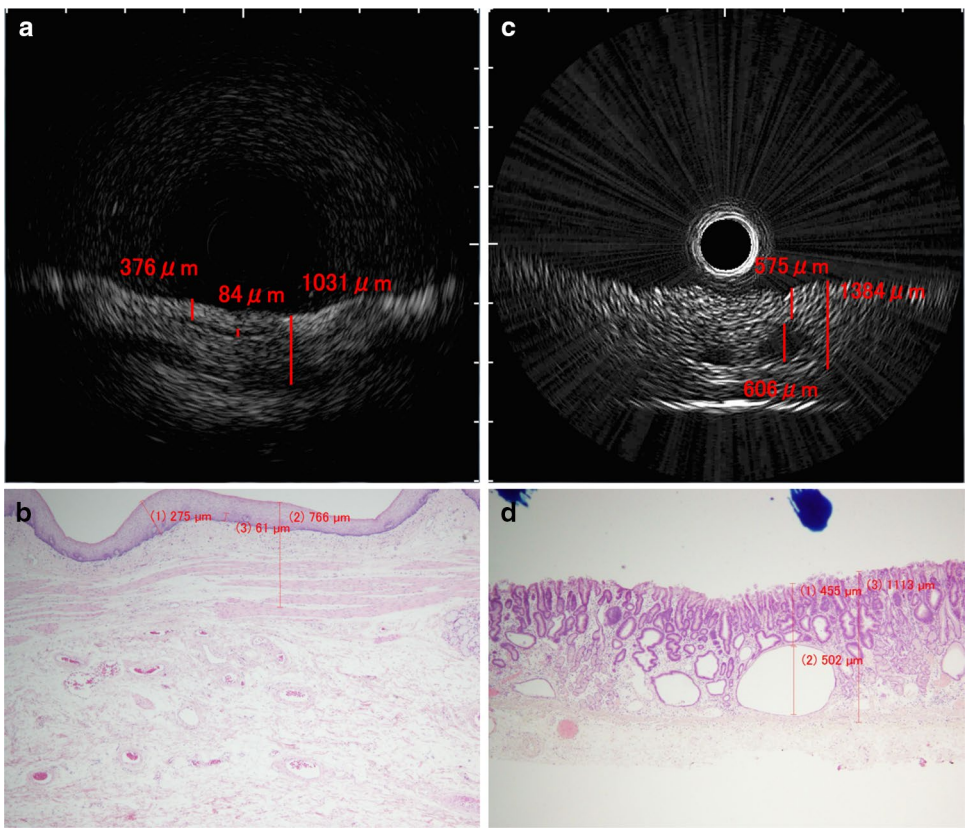

Fig. 4 Measuring the length of structure and layer. Normal esophagus, surgery **a** EUS; **b** hematoxylin and eosin, mag.×40. Reduction rate was 73%. Gastric adenoma, ESD. **c** EUS; **d** hematoxylin and eosin, mag.×40. Reduction rate was 80%

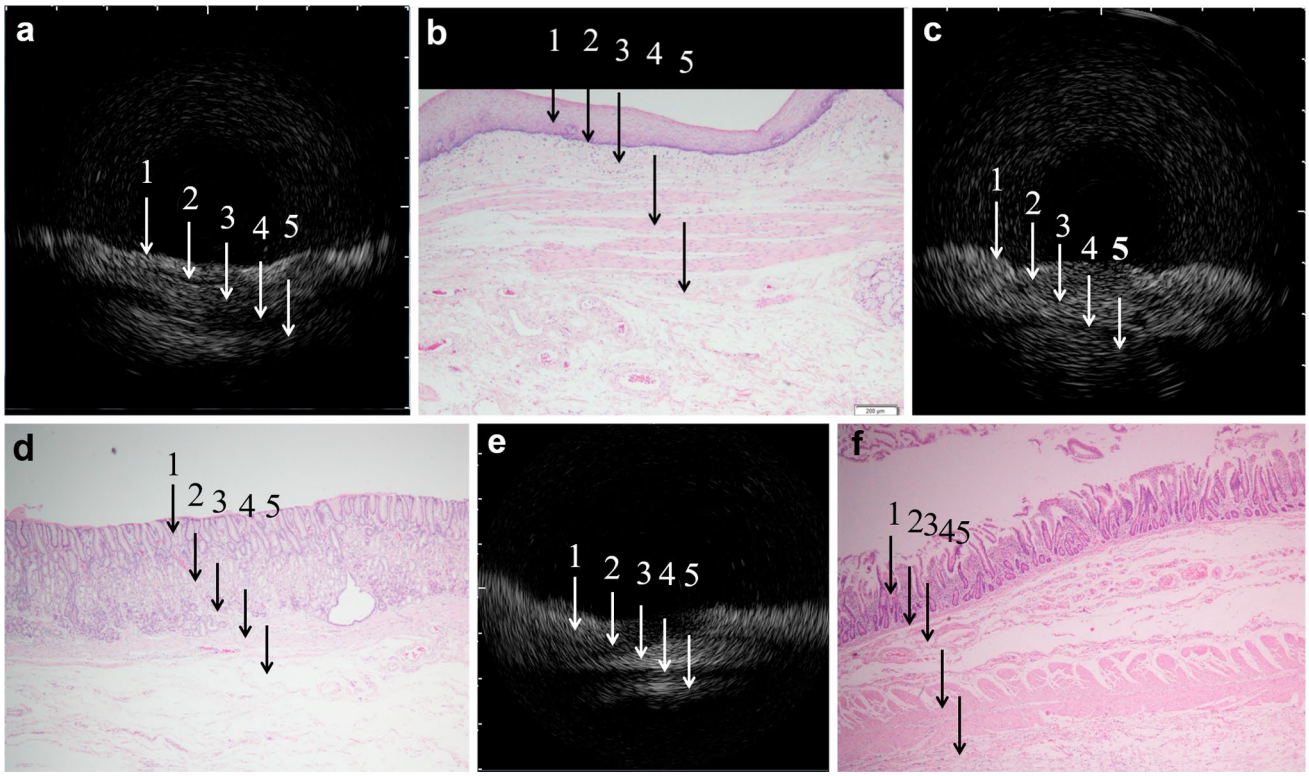

Fig. 5 Normal tissue. Esophagus **a** EUS; **b** hematoxylin and eosin, mag.×40, 1 (high echoic), EP; 2 (low echoic), basement of EP; 3 (high echoic), LPM; 4 (low echoic), MM; 5 (high echoic), SM, Stomach, **c** EUS; **d** hematoxylin and eosin, mag.×40, 1 (high echoic), upper M; 2 (low echoic), middle M; 3 (high echoic), lower M; 4 (low echoic), MM; 5 (high echoic), SM. Duodenum, **e** EUS; **f** hematoxylin and eosin, mag.×40, 1 (high echoic), M; 2 (low echoic), MM; 3 (high echoic), SM; 4 (low echoic), MP; 5 (high echoic), S. *EP* epithelium, *LPM* lamina propria mucosae, *MM* muscularis mucosae, *SM* submucosa, *MP* muscularis propria, *S* serosa

fibrosis in the pathological specimen, and the layer structure could not be determined by EUS either. In a case of advanced cancer, MM could not be seen because MM itself had disappeared due to tumor infiltration. MM could be visualized in all cases except the above two cases.

Discussion

The 60-MHz high-frequency probe was able to detect the layers of the gastrointestinal wall, including the MM, in the esophagus, stomach, and duodenum of all specimens included in this study. To the best of our knowledge, this is the first report of the use of a 60-MHz high-frequency probe as previous studies have only reported the use of probes up to 30 MHz.

In this study, the MM was depicted in all specimens, except in two specimens in which the layer was obscured by scarring or tumor infiltration. The visualization rate of the MM of the stomach is reported to be 50% using a conventional 20-MHz miniature probe, which means that it is not easy to depict the MM [9]. Improved visualization of the MM provides detailed images of the layered structure of the gastrointestinal tract up to the SM that may be used to diagnose tumor invasion. The depiction of delicate structures using conventional probes is challenging due to image resolution. In this study, dilated ducts and esophageal glands could be visualized in some specimens, indicating that more details of the layered structures can be visualized when using a high frequency. This allows for a more accurate evaluation of the depth of tumor invasion. However, as the visualization range is limited to 2 mm, the results of this study suggest that high-frequency EUS imaging with a 60-MHz probe may be more appropriate in organs with thin walls, such as the esophagus and duodenum, than in the stomach. Moreover, tall, elevated lesions and deeply depressed ulcerative lesions might be difficult to visualize with the 60-MHz probe due to the visualization range.

An accurate diagnosis of tumor infiltration near the MM is crucial to determine an appropriate endoscopic treatment. White light observation, image-enhanced magnifying endoscopy, and EUS are used to diagnose the depth of invasion in patients with gastrointestinal cancer [6, 7]. White light observation has been reported to have an accuracy of 68.1% in diagnosing the invasion depth of early gastric cancer in M/SM1 and an accuracy of 47.5% in diagnosing the invasion

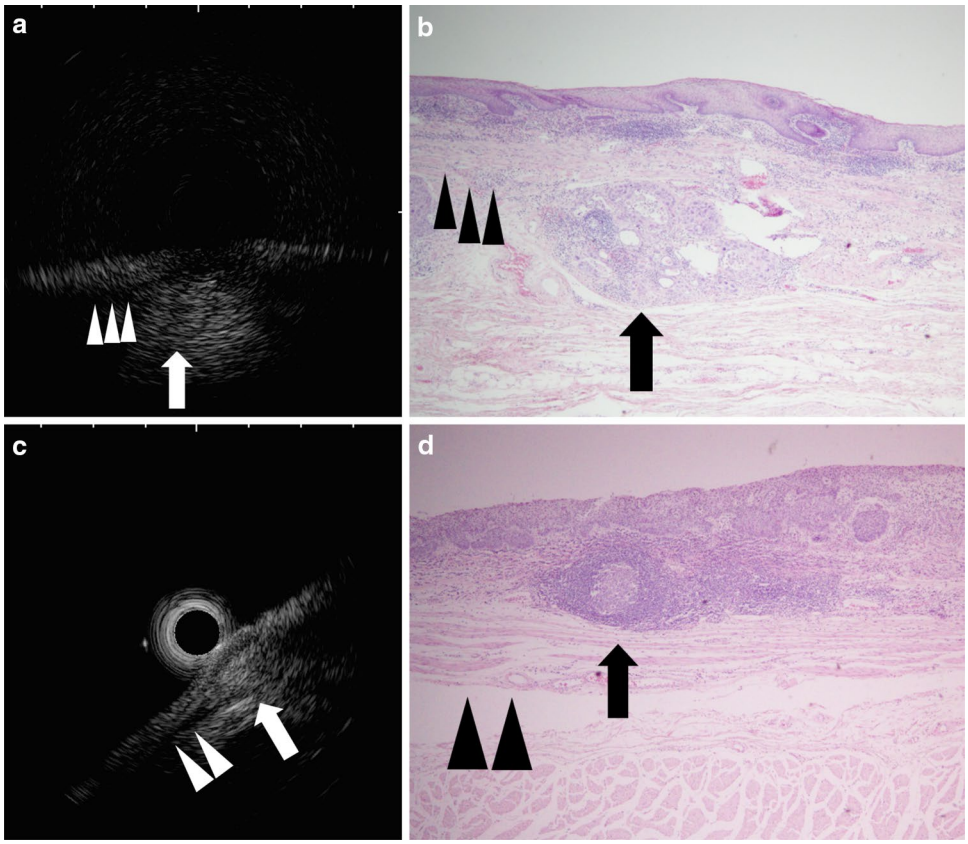

Fig. 6 Esophageal cancer. Case 1. **a** EUS; **b** hematoxylin and eosin, mag.×40. Arrowheads indicate the muscularis mucosae, and the arrow indicates cancer within the submucosa. Case 2. **c** EUS; **d** hematoxylin and eosin, mag.×40. Arrowheads indicate the muscularis mucosae, and the arrow indicates cancer within the layer

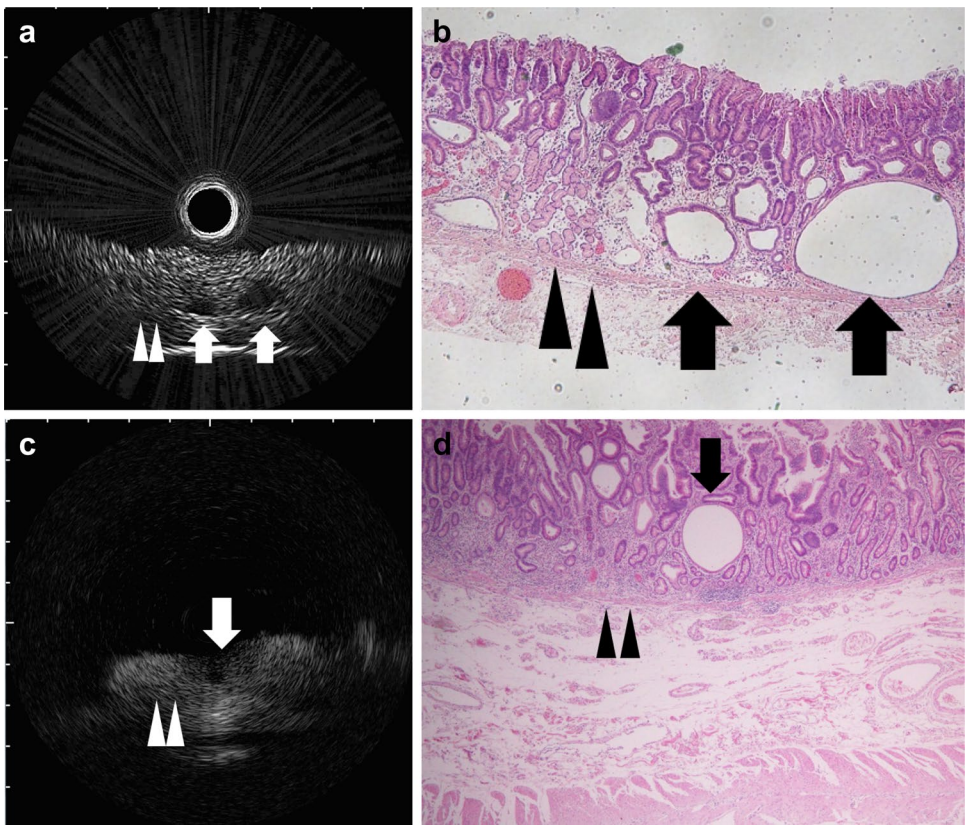

Fig. 7 Gastric adenoma. **a** EUS; **b** hematoxylin and eosin, mag.×40. Arrowheads indicate the muscularis mucosae, and the arrows indicate dilated ducts within the mucosa. Duodenal adenoma. **c** EUS; **d** hematoxylin and eosin, mag.×100. Arrowheads indicate the muscularis mucosae, and the arrow indicates dilated ducts within the mucosa

depth of early gastric cancer in SM2 or deeper [20]. When combined with EUS using a 20-MHz probe, the accuracy of the invasion depth of gastric cancer improved to 74.4% in M/SM1 and 73.7% in SM2 [20]. In contrast, the accuracy of white light observation for diagnosing the depth of invasion in patients with superficial esophageal cancer was 94% in the EP/LPM, 66–74% in the MM/SM1, and 61–74% in the SM2/SM3, for an overall accuracy of 85–89%. Image-enhanced magnifying endoscopy has been reported to have an accuracy of 68.6% for magnifying endoscopic classification of the Japan Esophageal Society in the MM/SM1, which is lower than its accuracy for classifying esophageal cancer in the EP/LPM (95%) or the SM2/SM3 (92.9%). In addition, the accuracy of EUS using a 20-MHz probe for esophageal cancer was 81% in the EP/LPM, 60% in the MM/SM1, and 90% in the SM2/SM3 [21].

EUS can diagnose gastric cancer in the M/SM1 and SM2 or deeper layers, which are the boundaries of endoscopic treatment. However, EUS is not able to sufficiently diagnose invasion of superficial esophageal cancer to the MM/SM1 level, which affects the endoscopic treatment indications of superficial esophageal cancer [22]. The 60-MHz probe improves the diagnosis of the depth of invasion as it enhances the boundary region between mucosal cancer and submucosal cancer, which has been reported as a limitation when determining the depth of invasion.

In this study, the layers of the esophagus, stomach, and duodenum were visualized using a 60-MHz high-frequency ultrasound probe. However, the 2-mm depiction range of the probe only allows for the delineation of the esophagus and stomach up to the SM, which results in five distinguished layers in the esophagus, stomach, and duodenum. With a 20-MHz probe, the esophagus can be visualized in seven to nine layers, with the third of seven layers corresponding to the SM layer. In contrast, Murata et al. reported that the first and second layers observed in the esophagus were the mucosal epithelium, the third layer was the LPM, the fourth layer was the MM, the fifth layer was the SM, the sixth to eighth layers were the MP, and the ninth layer was the adventitia when using a 30-MHz miniature probe [17]. The layered structure of the esophagus visualized with the 60-MHz probe was considered to be similar to the nine-layer structure. When the stomach is visualized in five layers with a 20-MHz probe, the first and second layers correspond to the M layer, and the third layer corresponds to the SM. At 60 MHz, the fourth layer of the stomach corresponds to the MM and the fifth layer to the SM; therefore, distinguishing the MM in the stomach using the 60-MHz probe is feasible. On the other side, the gastric mucosa was depicted as a three-layered structure, suggesting that the differences in the structures of the foveolar epithelium, the proliferative zone, and the fundic gland may have been recognized as the differences in echo-density. When the 20-MHz probe is used to visualize the layers of the duodenal wall, the first and second layers are the M layers, the third layer is the SM, the fourth layer is the MP, and the fifth layer is the S. Using the 60-MHz probe, the entire duodenal layer can be visualized in five layers, which is considered to be the whole thickness of the duodenal wall. The difference from the case of 20 MHz is that the first layer corresponds to the M layer and the second layer corresponds to the MM layer. Therefore, distinguishing the MM in the duodenum using the 60-MHz probe is probable.

This study is novel and unique; however, it has a few limitations. First, the number of cases was small. Second, it is a single-center study. Third, this probe cannot be used in vivo. In vivo scanning through the endoscope's working channel requires improvements to the probe, including improved durability and image quality. Fourth, the specimen study used only a 60-MHz miniature probe. This specimen study did not compare images depicted by the 60-MHz miniature probe with those acquired with a 20-MHz miniature probe or 7.5-MHz conventional convex-type endoscopic ultrasonography. Therefore, it is not possible to directly compare the visualizations of MM. This point will need further consideration in future research. Fifth, a method to compare the drawn image and the pathological image is needed. In a previous report, EUS images and pathological images were compared for evaluation. In this study, we excised the visualized area and evaluated the layer structure by measuring the drawn image and the pathological image. The reduction rate of the EUS image and the pathological image after fixing the specimens in 10% formalin was approximately 80%, which is consistent with those of previous reports [23]. As a more direct layer comparison method, a method of inserting nylon threads into each layer of the stomach wall of the specimen and comparing the EUS image with the pathological image to identify the layer structure has been reported [24]. Further consideration is needed to improve the image-matching accuracy.

As this study is a feasibility study using a prototype device, a new study design is needed based on the results of this study. To evaluate the usefulness of the 60-MHz probe, it is necessary to visualize normal tissue and tumor tissue and compare the invasion depth diagnostic ability under the same conditions as conventional devices.

Conclusion

The 60-MHz probe allows for the visualization of the muscularis mucosae and the layer structure up to the submucosa, which improves the ability to diagnose the depth of early cancer invasion of the upper gastrointestinal tract, allowing for more appropriate treatment methods.

Acknowledgements None

Declarations

Conflict of interest The authors borrowed a prototype 60-MHz miniature probe from Fujifilm Corp. (Tokyo, Japan) to collect practical data. Fujifilm Corp. had no role in the design, practice, or analysis of this study.

Ethical approval Informed consent to the protocol was obtained from all patients. This study was approved by our institutional ethics committee for human research.

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