In vivo histopathological assessment of the muscularis propria in achalasia by using endocytoscopy (with video)

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**Background:** The histopathology of the muscularis propria (MP) is unknown in patients with achalasia. Endocytoscopy (EC) was developed as an ultra-high magnification endoscopy, and the submucosal tunnel created during peroral endoscopic myotomy (POEM) not only provides access to the MP but also enables subsequent endoscopic assessment of the MP.

**Patients and Methods:** In seven patients with achalasia (mean ±SD: 35 ± 18.1 years; men:women, 4:3) who underwent POEM (myotomy length: 12± 2.2 cm), subsequent EC examination was performed from the mid-esophagus to the gastric side. EC images were compared to the results of histopathologic examination (two biopsies from the mid-esophagus and lower esophageal sphincter), which was the standard.

**Results:** In all patients, favorable EC images were obtained, and spindle-shaped smooth muscle cells were detected. In our series, we observed no notable features such as atrophy or hypertrophy of smooth muscle cells. In addition, the EC assessment was consistent with the results of biopsy. No complications were encountered during any of the procedures.

**Conclusion:** In a clinical setting, real-time assessment of the MP using EC is feasible. This technique may play an important role in determining the pathology of achalasia and other diseases that affect gastrointestinal function.

**Introduction**

Ultra-high magnification endoscopy, also known as endocytoscopy (EC) or endomicroscopy, enables assessment of the muscularis propria (MP) or myenteric plexus in vivo through a submucosal tunnel [1 – 3]. This technique is expected to be applicable for clinical use for the treatment of neuromuscular diseases because the nidus of such diseases is under the mucosa, a location that is difficult to assess using conventional endoscopy. Achalasia is an idiopathic esophageal motility disorder characterized by a lack of peristalsis in the esophageal body and absent or incomplete relaxation of the lower esophageal sphincter (LES) [4 – 5]. The novel procedure, peroral endoscopic myotomy (POEM), has become one of the best treatment options for achalasia because it is safe, offers long-lasting symptom control, and is less invasive than surgery [6 – 8]. Moreover, the submucosal tunnel created during POEM allows insertion of the endoscope under the mucosa and access to the MP. During POEM, thick muscle at the middle to lower esophagus, as well as thin muscle at the LES area, can be identified (Fig. 1), although there are variations among patients with achalasia [9, 10].

The histopathology of achalasia has been insufficiently investigated because of the low prevalence of this condition, and because the mainstream treatment for achalasia has been balloon dilatation, which does not allow tissue sampling. Surgical myotomy may allow access to the MP at the LES but, because of the limited treatment window (difficult to pull the mid-esophagus into the abdominal cavity), using surgical myotomy to assess the mid-esophagus is technically difficult. The approach to and examination of the esophageal MP was difficult until the introduction of POEM.

The purpose of the current study, therefore, is to determine the feasibility of EC for real-time histopathologic examination of the MP in a clinical setting, and to provide more knowledge about the histopathologic features of esophageal tissues features of esophageal tissues in achalasia.

**Patients and Methods**

**Patients**
This study was performed at Showa University Northern Yokohama Hospital, a tertiary referral center in Japan. Enrollment began in December 2009.
2013 and patients with consecutive achalasia who were candidates for POEM were recruited for this study. Patients younger than 18 years and those whose health was not favorable for enrollment because of severe comorbidity in organs such as the heart or lungs were excluded from this study. In instances where full-thickness myotomy was performed, EC examination was not performed, and these patients were also excluded from the analysis. The current study was approved by the Institutional Review Board (No.1311-05). Written informed consent was obtained from all participants, and the study was conducted according to the Declaration of Helsinki.

**Peroral endoscopic myotomy**

POEM was performed following the technique described by Inoue [6]. Before the procedure, esophageal contents were cleared by endoscopic suction using a 3.7-mm channel endoscope (GIF-1T240; Olympus Co., Tokyo, Japan) under intravenous anesthesia to prevent aspiration caused by intubation. Under general anesthesia with positive pressure ventilation, the endoscopic procedure began with CO₂ insufflation. After submucosal injection at the level of the mid-esophagus, a 2-cm longitudinal mucosal incision was created as the point of entry. A one-third circumferential submucosal tunnel was created from entry down to the LES and into approximately 2–3 cm of the gastric side. Dissection of the circular muscle (from 2 cm distal to the mucosal entry) was performed at the center of the submucosal tunnel. After myotomy, a totally relaxed C (EGJ) was confirmed by inserting an endoscope into the natural lumen down to the gastric side, and by a retroflex view from the gastric side to EGJ (Fig. 2). The mucosal entry was closed with hemostatic clips.

**Endocytoscopy**

All EC examinations were performed using the integrated-type endocytoscope GIF-Y0002 (prototypes from Olympus Co.). GIF-Y0002 has one lens that can consecutively increase the magnification from the conventional endoscopy level to 380 × magnification (tissue field of view, 700 × 600 μm) using a hand lever. The GIF-Y0002 allows gradual magnification at the center of the monitor, thus ensuring accurate localization of the area being viewed. For EC, a mixture of 0.1% methylene blue was used to stain the tissues and to obtain images similar to those obtained in conventional pathology examination. Methylene blue staining reveals details of cell structure, including nuclei and cytoplasm.
Study procedure

EC examination was performed after POEM and prior to closure of the mucosal entry site. After staining with 2 mL of methylene blue, the head of the endocytoscope was moved closer to the surface of the MP. The EC examination was started from the proximal 2 cm of circular muscle through remnant circular muscle into the gastric side. Longitudinal muscle was also examined. Two biopsy specimens were obtained from the proximal circular muscle and at the LES area (high-pressure zone) for histopathological assessment as confirmation of in vivo diagnosis. EC findings were assessed according to the previous in vivo report and histopathology reports [1, 11, 12]. EC findings of the MP were classified as: atrophy, normal, or hypertrophy. Biopsy specimens were assessed by a pathologist blinded to EC images.

Results

Seven patients with achalasia who underwent POEM were enrolled in this study and underwent concomitant EC examination. The mean (±SD) age of the participants was 35.0±18.1 years, and the study population consisted of four male and three female patients. The duration of achalasia symptoms was 5.5±7.5 years, and ranged from 3 to 20 years. According to Chicago classification criteria [13, 14], manometric findings were classified as type I in one patient and type II in four patients; in two patients, manometry was not completed because of difficulty in inserting the catheter through the LES. The total length of the endoscopic myotomy was 12±2.2 cm with a mean of 10±2.0 cm in the esophagus and 3.0±0.69 cm in the stomach (Table 1).

Favorable EC images were obtained, and spindle-shaped smooth muscle cells were observed. A strong staining nucleus, surrounding cell bodies, and vessels in the muscular layer were visualized. However, none of these structures showed particularly notable changes, that is, non-atrophy and non-hypertrophy of the MP (Fig. 3). Moreover, EC findings were consistent with biopsy results (Fig. 4). In other words, changing patterns such as atrophy or hypertrophy were not identified on the biopsy specimens. EC and biopsy were performed within 10 minutes with no complications. On postoperative day 2, a liquid diet was started, and on day 4, all the patients were discharged on a normal diet.

Discussion

To our knowledge, this is the first study to report in vivo histopathology of the MP in patients with achalasia. Our results offer the following propositions. First, EC can be used for in vivo real-time assessment not only for intestinal mucosa but also for MP via a submucosal tunnel created after POEM. In POEM for achalasia,
real-time histopathological assessment of MP may be helpful to identify the morbid lesion preoperatively, although no atrophy or hypertrophy was identified in our small cohort. The intermittent and time-consuming biopsy method, which also includes adverse event risks such as bleeding, cannot substitute for the continuous and real-time EC assessment. Furthermore, if EC can be performed by resecting the mucosal/submucosal layer using endoscopic mucosal resection/endoscopic submucosal dissection, EC is potentially effective for the diagnosis of other disorders with a nidus located in the MP. Second, the endocytoscopic morphology of the MP showed no evidence of hypertrophy or atrophy of smooth muscle cells despite a thick or thin appearance on a conventional endoscopic view, and the EC findings were confirmed by histology. This result suggests that the thick or thin appearance of MP in achalasia is caused by the changing histopathology rather than hypertrophy or atrophy, or that such change requires long-term symptoms. Previous studies have examined muscle biopsies from patients with achalasia treated at an earlier stage of disease. These studies have detected intact MP in these patients who had a shorter duration of symptoms and a non-dilated esophagus [15–17], findings that are almost consistent with our results. This study has several limitations. First, the staining technique used in this series allowed the evaluation of cell structure, cytoplasm, and nuclei alone. The nerve plexuses were not visualized in this setting because of a lack of neuron-specific fluorescent stain available for safe use in humans. Development of stains and methods to evaluate stain toxicity and long-term effects are essential. In the current study, the nerve plexus was not identified by biopsy, probably because the tissue sampling should be nonconsecutive, and with a small specimen. It is expected that EC will help identify the nerve plexus in clinical settings.

Competing interests: None.

References
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