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ORIGINAL ARTICLE - GASTROENTEROLOGY (CLINICAL)

Beige mucosa observable under narrow-band imaging indicates the active sites of eosinophilic esophagitis

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Key words
beige mucosa, diagnosis, endoscopic finding, eosinophilic esophagitis, narrow-band imaging.

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Abstract

Background and Aim: The majority of patients with eosinophilic esophagitis (EoE) are likely to have observable features under narrow-band imaging, namely beige mucosa. However, the histological features and clinical implications of beige mucosa have not been investigated. The aim of this study was to determine whether beige mucosa could serve as an endoscopic marker for predicting active inflammatory sites of EoE.

Methods: We retrospectively analyzed both the narrow-band images and biopsied specimens of 77 esophageal lesions from 35 consecutive patients with EoE. We divided these specimens into two groups: target biopsied specimens from beige mucosa (beige group) and specimens biopsied from non-beige mucosa (non-beige group). The number of eosinophils per high-powered field, thickness of the superficial differentiated cell layer, and depth of the hemoglobin component from the surface layer were compared between the two groups.

Results: Forty-four out of the 45 specimens were diagnosed as histological active lesions in the beige group. The sensitivity, specificity, and overall accuracy of beige mucosa in predicting EoE activity were 97.8%, 96.9%, and 97.8%, respectively. Compared with the non-beige group, specimens in the beige group had a significantly thinner superficial differentiated cell layer.

Conclusions: Beige mucosa is associated with thinning of the normal superficial differentiated cell layer, and these histological changes in the active inflammatory sites of EoE could be recognized endoscopically as color differences. Beige mucosa may serve as an endoscopic indicator for predicting the histological activity of EoE.

Introduction

Narrow-band imaging (NBI) is a non-invasive technique that uses reflected light and reportedly improves the detection of various epithelial changes in the superficial layers.[1] It was reported that most patients with eosinophilic esophagitis (EoE) are likely to have observable features under magnified NBI observation, namely beige mucosa, dot-shaped interpapillary capillary loops, and absence of submucosal (cyan-colored) vessels.[2] Beige mucosa has been reported as one of the particular features of magnified NBI observation, but in daily practice, we have often encountered EoE patients with beige mucosa visible in the distance view without magnification (Fig. 1). In addition, we noticed that most of the biopsy specimens taken from the beige mucosa had eosinophil infiltration. The short-wavelength light of 415 nm used in NBI only penetrates the superficial layers of tissue and is strongly absorbed by hemoglobin (Hb).[1] We hypothesized that histological changes in the superficial epithelium in the active inflammatory sites of EoE could be recognized endoscopically by NBI as changes in color (Fig. 2). Accordingly, this study was undertaken to determine the accuracy of the beige mucosa as an endoscopic marker for predicting active inflammatory sites of EoE and to clarify the histological changes responsible for the development of beige mucosa.

Materials and methods

Patients and data collection. This was an observational study conducted at Kawasaki Medical School General Medical Center, Okayama, Japan, and Sakaide City Hospital, Kagawa, Japan, between April 2017 and March 2019.

We retrospectively enrolled 35 consecutive adult patients with EoE who underwent endoscopic NBI evaluation as part of their clinical care. EoE was diagnosed by international consensus criteria, which included symptoms of esophageal dysfunction and esophageal biopsy findings of esophageal mucosal eosinophilia [≥ 15 eosinophils per high-power field (HPF)].[3] We obtained targeted specimens from the areas involved in EoE-related changes (such as furrows or exudates) under white-light imaging (WLI) observation.[4] If no findings were observed, specimens were collected from the normal-appearing proximal and distal

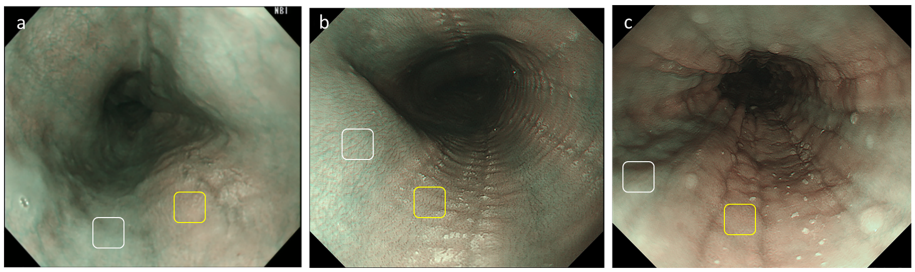

Figure 1 Beige mucosa. Representative endoscopic findings of the beige mucosa, defined as light brownish areas on narrow-band imaging (yellow square). Non-beige mucosa, defined as areas of light green epithelium (white square).

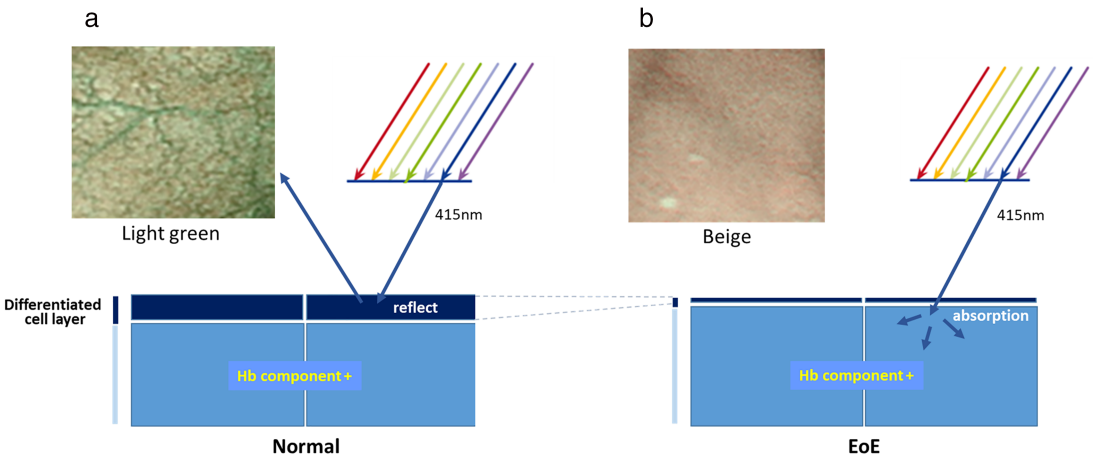

Figure 2 Schematic representation of the interactions between tissue and short wavelength of 415 nm (blue bands) when using narrow-band imaging: (a) in normal epithelium, the blue bands are reflected by the surface of the differentiated squamous cell layer. (b) In active inflammatory sites of EoE, the blue bands are allowed to contact the Hb component due to the loss or thinning of the superficial differentiated squamous cell layer. EoE, eosinophilic esophagitis; Hb, hemoglobin.

esophagus, and at least four specimens were taken by WLI. After conventional WLI observation, NBI observation was performed, and at least two biopsies were taken while checking for the presence of beige mucosa.

Assessment of endoscopic findings. High-resolution endoscopes used in this study were either the GIF-260 series or the GIF-290 series (both from Olympus Medical Systems, Tokyo, Japan). The endoscopic images were randomly reviewed by two expert endoscopists (with 15 and 22 years of experience, respectively) to confirm the endoscopic findings in the biopsied lesions without knowing each patient's clinical information. Discrepancies were resolved by consensus.

Beige mucosa was defined as mucosa that appeared beige in color compared with normal mucosa, which has a light green color, based on a previous report (Fig. 1).[2] Non-beige mucosa was defined as areas of light green epithelium that were confirmed not to have beige mucosa under NBI (Fig. 1). Additionally, the color analysis was performed with Adobe Photoshop CS4 (Adobe Systems Inc., San Jose, California, United States). The color difference (ΔE) was calculated using the CIELAB color space, a three-dimensional color space that comprises a black-white axis (L*), a red-green axis (a*), and a yellow-blue axis (b*) and associates color perception with colorimetric values.[5] The color difference (ΔE*) between beige mucosa and the surrounding normal mucosa was calculated according to the equation: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. Moreover, the ΔE* values were converted into National Bureau of Standards (NBS) units using the following formula: NBS units = ΔE* × 0.92. The NBS defines the units of color difference as follows: 0–0.5 = trace; 0.5–1.5 = slight; 1.5–3.0 = noticeable; 3.0–6.0 = appreciable; 6.0–12.0 = much; ≥ 12.0 = very much.[6]

Other endoscopic findings related to EoE (e.g., edema, rings, exudates, furrows, and strictures) of each biopsy site under the WLI observation were evaluated based on the Endoscopic Reference Score (EREFS).[4] The EREFS classification system grades the severity of edema (0–2), rings (0–3), exudates (0–2), furrows (0–2), and strictures (0–1). Total EREFS is calculated by combining individual values; it ranges from 0 to 10.[4]

Esophageal biopsies and histologic evaluation. A total of 215 biopsy specimens (75 from the proximal side of the

esophagus and 140 from the distal side) were collected from 35 patients, of which 77 biopsy specimens were collected (25 from the proximal side of the esophagus and 52 from the distal side) with a focus on color changes under NBI observation. We divided these 77 specimens into two groups: target biopsy specimens from beige mucosa (beige group) and specimens biopsied from non-beige mucosa (non-beige group). We first evaluated the EoE histological activity in biopsy specimens from each region designated as an endoscopic finding of EREFS. Then, the EoE histological activity was evaluated in the target biopsy specimens of beige and non-beige mucosa by NBI observation.

The following histological features were also evaluated based on previous reports[7]: basal layer hyperplasia (expansion of the basal zone by $>$ 25% of the epithelial height), spongiosis (also termed dilated intercellular spaces), eosinophilic microabscesses (defined as clusters of $\geq$ 4 eosinophils), and lamina propria fibrosis (evaluated if lamina propria was present in the specimen). To clarify the histological changes responsible for the development of beige mucosa, the presence of Hb components in the superficial layer was evaluated. This evaluation was performed because 415-nm (blue) light is easily absorbed by Hb protein and penetrates only the superficial layer of the mucosa[1]; therefore, the presence or absence of Hb components in the superficial layer is considered to be significantly related to the color change. The thinning of the superficial differentiated cell layer, which has been reported as an important pathogenic factor for the brown appearance of esophageal neoplasms under NBI observation,[8] was also evaluated and compared between the two groups (Fig. 2).

Histological assessments were conducted by one of the authors who was blinded to the endoscopic images. The peak count of intraepithelial eosinophils/HPF (×400) was determined in the area of highest density of eosinophils by the most densely populated. Histological active EoE was defined by the presence of $\geq$ 15 eosinophils/HPF in at least one field in this study. For the histological evaluation of the basal cell hyperplasia, thickness of the superficial differentiated cell layer, and the depth of the Hb component from the surface layer, only specimens that were cut perpendicular to the epithelial surface and contained the entire mucosal epithelial layer were evaluated, because the values depended on how the specimens were sliced. The three most representative sites were measured, and average values were calculated.

The presence of Hb protein was evaluated using anti-Hb antibody (Ab). The specimens were incubated overnight at 4°C with a primary polyclonal antibody to human Hb (Bethyl Laboratories, Inc., Montgomery, TX, USA). Specimens were then incubated with a labeled polyclonal rabbit anti-goat biotinylated antibody (Dako, E0466) for 30 min and LSAB2 System-HRP kit (Dako, K0675) containing biotinylated link antibody and streptavidin-conjugated HRP for 10 min in succession. Between each step, the slides were washed in 0.05-M phosphate buffer solution at pH 7.6. DAB (3,3′-diaminobenzidine tetrahydrochloride) was used for the staining. In the final step, slides were counterstained with hematoxylin.

Statistical analysis. Statistical analyses were carried out using the Mann–Whitney *U*-test; Fisher's exact test was also used when appropriate. Clinical and demographic variables were described with the use of frequencies and percentages for categorical variables and mean and standard deviation or median and interquartile range for quantitative variables, as appropriate. *P* values $<$ 0.05 were considered to indicate statistical significance. All statistical analyses were performed using SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA).

The study was approved by the Kawasaki Medical School Ethics Committee (approval no. 3965). All samples were obtained with the written informed consent of patients prior to their inclusion in the study, in accordance with the provisions of the Helsinki Declaration.

Results

Patient characteristics. The mean age of the 35 patients with EoE enrolled in the study was 44 years (range, 25–68 years). The patient group comprised 23 men and 12 women. Twenty-two of the 35 patients with EoE had allergic conditions, such as hay fever (n = 14), asthma (n = 5), food allergy (n = 4), or atopic dermatitis (n = 2) (including duplicates). Of the 35 patients, 10 patients were on proton pump inhibitor therapy at the time of endoscopy at our institution (Table 1).

Among the 35 patients, "Edema" was seen in 33 (94.3%), "Rings" in 13 (37.1%), "Exudate" in 11 (31.4%), and "Furrows" in 31 (88.5%), and no patient had "Stricture", with a mean EREFS of 2.96 ± 0.87. Beige mucosa was seen in 34 patients (97.1%) (Table 1). Most patients had both morphological and color changes (beige mucosa) (Fig. 3a), although two patients had only color changes (Fig. 3b).

Diagnostic ability of each finding of EREFS to predict EoE activity. Of the 138 specimens collected under WLI observation, 20 specimens were from areas of "Edema," 13 were from "Rings," 22 were from "Exudate," 62 were from "Furrows," and 21 were from the normal-appearing esophagus.

Table 1 Clinical characteristics of the surveyed patients with EoE

Patients, *n*	35
Age and sex	
Mean (range), years	44 (25–68)
Men, *n* (%)	23 (65.7)
Concomitant allergic diseases, *n* (%)	22 (62.8)
Hay fever, *n* (%)	14 (40)
Asthma, *n* (%)	5 (14.3)
Food allergy, *n* (%)	4 (11.4)
Atopic dermatitis, *n* (%)	2 (5.7)
PPI user, *n* (%)	10 (28.6)
Endoscopic findings (WLI)	
Edema, *n* (%)	33 (94.3)
Ring, *n* (%)	13 (37.1)
Exudates, *n* (%)	11 (31.4)
Furrows, *n* (%)	31 (88.5)
Stricture, *n* (%)	0 (0)
Normal, *n* (%)	2 (5.7)
Endoscopic findings (NBI)	
Beige mucosa, *n* (%)	34 (97.1)

EoE, eosinophilic esophagitis; PPI, proton pump inhibitor.

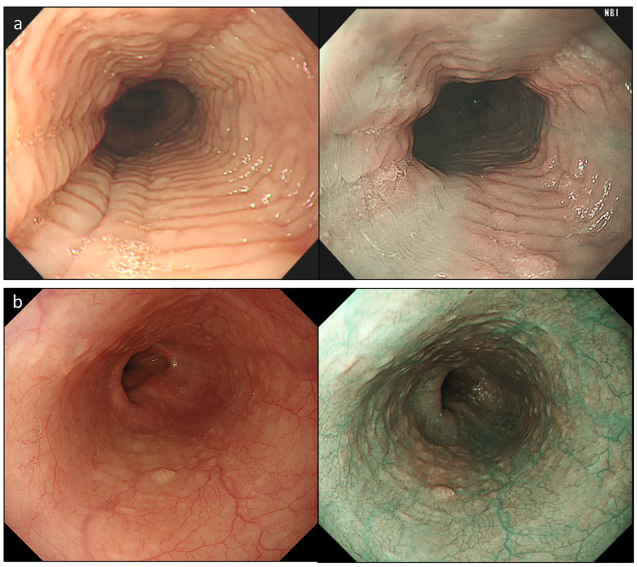

Figure 3 Endoscopic images of eosinophilic esophagitis: (a) morphological changes can be seen in both white-light imaging and narrow-band imaging (NBI), and the inflammatory area can be recognized by the color change of the beige mucosa under NBI observation. (b) Characteristic endoscopic findings of eosinophilic esophagitis, such as furrows, rings, or exudates, were not clearly observed, but beige mucosa was detected by NBI.

Specimens from each endoscopic finding of EREFS were histologically active (> 15 eosinophils) in 70% (14/20) for "Edema," 39.4% (13/33) for "Rings," 90.9% (20/22) for "Exudate," and 64.5% (40/62) for "Furrows." Among 21 specimens biopsied from normal-appearing esophagus, two specimens (9.5%) had histological activity (Table 2).

Accuracy of the beige mucosa to predict histological activity. Of the 77 specimens collected under NBI observation, 45 were from beige mucosa (beige group) and 32 were from non-beige mucosa (non-beige group). The area designated as beige mucosa fully met the criteria to be recognized as a different color with a mean value of 14.31 (range: 8.31–27.01) in the objective evaluation of color difference by NBS units.

Specimens biopsied from beige mucosa were more histologically active lesions (> 15 eosinophils) than those biopsied from non-beige mucosa (97.8% *vs* 3.1%, $P < 0.01$) (Table 2). The sensitivity, specificity, and overall accuracy of beige mucosa in predicting EoE activity were 97.8%, 96.9%, and 97.8%, respectively.

Other histological findings also showed significant differences between the two groups, as follows: basal cell hyperplasia (69.7% *vs* 15.0%, $P < 0.01$), spongiosis (97.8% *vs* 9.4%, $P < 0.01$), and eosinophilic microabscesses (22.2% *vs* 0%, $P < 0.01$). The presence of lamina propria fibrosis was not shown because few samples contained lamina propria, which led to unstable accuracy.

Histological evaluation responsible for the development of the beige mucosa. Among the 77 specimens, 55 specimens (35 in the beige group and 20 in the non-beige group) were cut perpendicular to the epithelial surface and contained the entire mucosal epithelial layer.

Immunohistological images revealed a clear boundary between the anti-Hb Ab-positive and anti-Hb Ab-negative areas. In the beige group, Hb protein was present in the superficial layer of the tissue, while in the non-beige group, Hb protein was not present in the superficial layer of the tissue (Fig. 4). The distance from the surface layer to the Hb component was significantly smaller in the beige group [0 μm (0, 10) *vs* 70 μm (60, 88), $P < 0.01$] (Fig. 5a). Compared with the non-beige group, specimens in the beige group had a significantly thinner superficial differentiated cell layer [8 μm (0, 13) *vs* 70 μm (60, 102), $P < 0.01$] (Fig. 5b). The anti-Hb Ab-negative area was almost perfectly matched to the area with a normal superficial cell layer (Fig. 4).

Discussion

In this study, we revealed that the beige mucosa accurately reflected the histological active sites of inflammation and that the presence of extravascular Hb protein in the surface and loss or thinning of the normal superficial differentiated cell layer are involved in the color change.

The transmission of 415-nm light, which is strongly absorbed by Hb and only penetrates the mucosal surface, is dependent on Hb distribution and the epithelial surface structure.[1] Normal squamous epithelium with stratum granulosum reflects light well and thus appears light green in color under NBI.[8] When thinning of the superficial differentiated cell layer including the stratum granulosum has occurred, the 415-nm light does not reflect, but

Table 2 Target biopsy site and histological activity

	Endoscopic finding at biopsied sites						
	WLI					NBI	
	Edema	Ring	Exudates	Furrows	Normal	Beige mucosa	Normal (non-beige)
Total biopsied specimens, *n*	20	33	22	62	21	45	32
With histological activity (> 15 eosinophils), *n* (%)	14 (70)	13 (39.4)	20 (90.4)	40 (64.5)	1 (9.5)	44 (97.8)	1 (3.1)

NBI, narrow-band imaging; WLI, white-light imaging.

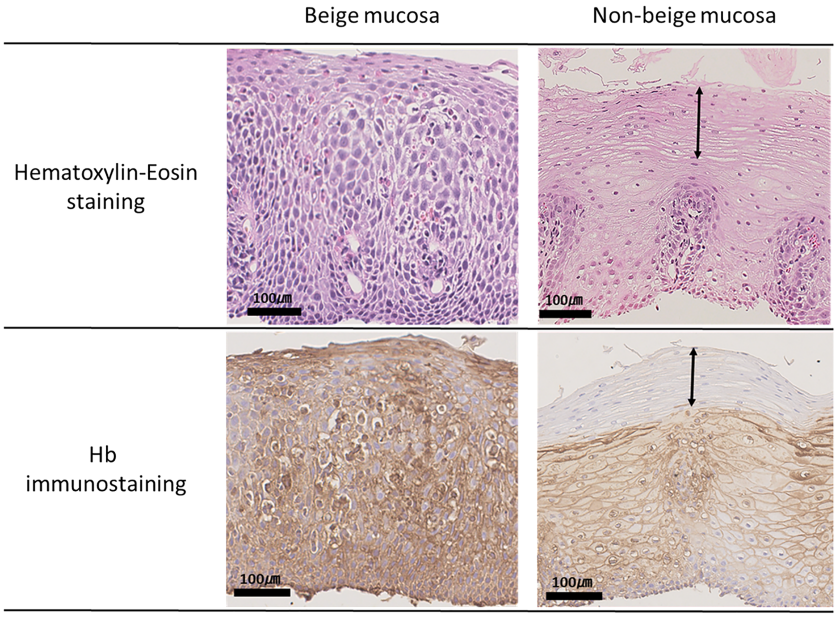

Figure 4 Representative immunohistological images of beige mucosa and non-beige mucosa stained with anti-human Hb antibody (anti-Hb Ab). Left image: Beige mucosa with no differentiated cell layer and intense positivity to anti-Hb Ab throughout the epithelium, including the superficial layer. Right image: Non-beige mucosa has a thick differentiated cell layer (black arrow), and this area is negative for anti-Hb Ab. Hb, hemoglobin.

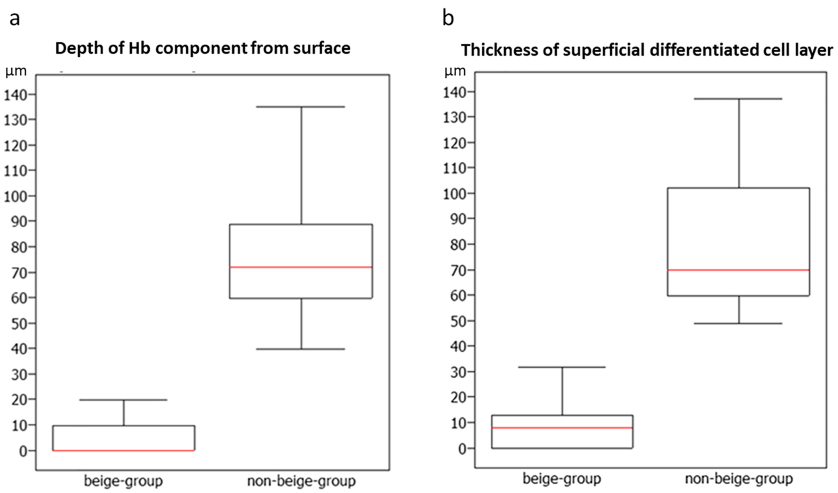

Figure 5 Box-whisker plots showing comparisons of the beige and non-beige groups regarding the (a) depth from the surface layer to the hemoglobin component and (b) differentiated cell layer thickness.

penetrates the surface and is absorbed by Hb during its optical path, making the mucosa appear darker in color.[8,9] Recently, several studies have reported that a loss of esophageal epithelial differentiation was a specific and integral part of the pathophysiology of EoE.[10,11] Therefore, we considered that loss or thinning of the superficial differentiated cell layer caused by the activity of EoE allows 415-nm light to contact the Hb component, resulting in a beige color change (Fig. 2).

In our study, the distance from the surface layer to the Hb component was shorter than 20 μm in all cases of beige mucosa, while the Hb component was found at a depth of 40 μm from the superficial layer in the shallowest specimens in the non-beige group (Fig. 5a). Further large prospective studies will be mandatory to determine the exact correlation between the depth of the Hb component and color change.

Minami *et al*. reported the presence of extravascular Hb components in esophageal squamous cell carcinoma, and further reported that the anti-Hb Ab-positive area was perfectly matched to the cancer area, while the surrounding noncancerous epithelium was negative for Hb staining.[12] However, our study showed positive Hb staining within the epithelium despite the noncancerous lesions, which suggests that the Hb component in the esophageal epithelium may not be a cancer-specific change (Fig. 4). There are few studies on the expression of Hb in esophageal epithelial cells, although the expression of Hb in non-erythroid cells such as alveolar epithelial cells and renal mesangial cells has been reported.[13,14] Epithelial–mesenchymal transition (EMT) is considered to contribute to esophageal remodeling and reverses with treatment in EoE.[15,16] EMT describes a series of events in which epithelia lose many epithelial characteristics, including polarity and tight

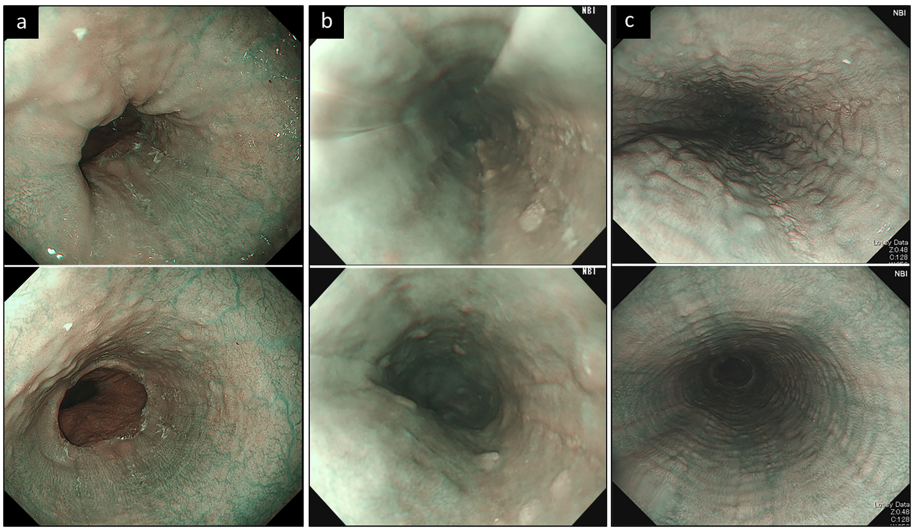

Figure 6 Representative endoscopic images of beige mucosa with narrow-band imaging under less air and standard air conditions. (a–c) Upper image: less air condition. Lower image: standard air condition.

junctions, and acquire properties of mesenchymal cells, including motility and loose cell adhesion.[17] EMT has also been reported to be involved in cancer invasion in the esophagus.[18] Further investigation will be required to assess the causes and mechanisms of Hb expression in esophageal epithelial cells.

According to a previous report, NBI did not improve the detection of typical endoscopic findings of EoE, including rings and furrows.[19] However, beige mucosa is a completely different finding from these morphological changes, and focuses on color changes. In this study, we found that 9.5% of biopsies taken from areas that did not show typical findings such as furrows or exudates under WLI and appeared to be normal esophagus showed histological activity. The evaluation of beige mucosa by NBI may be useful for recognizing inflammation in areas without morphological changes. In this study, as in previous reports,[20] among the various findings of EREFS, biopsy specimens from exudates were considered to be the most suitable sites for targeted biopsies due to their high potential for histological activity. However, exudates were found in only 31.4% of patients with EoE in this study. Notably, beige mucosa was found in 97.1% of patients with EoE. The beige mucosa reflects histological changes such as loss of squamous differentiation, which is the essence of the pathogenesis of EoE,[10,11] and seems to represent a suitable site for targeted biopsy.

The visibility of brownish area was reported to be higher with less air condition than with standard air conditions in a flat type esophagus squamous cell carcinoma.[21] Similar to brownish area, beige mucosa has also a tendency to appear more brownish with less air volume condition (upper part of Fig. 6). On the other hand, if the air volume is low, the field of view becomes narrower, the beige mucosa and the green normal mucosa do not fit in the same field of view, and the contrast between the beige mucosa and the normal mucosa is not clear, which may make it difficult to recognize color changes (Fig. 6c). In this study, we did not make any special adjustments to the air volume when evaluating beige mucosa. Controlling the amount of air is a very important factor for visibility, and the optimal observation conditions for evaluating of beige mucosa will be a subject for future study.

There are several limitations of this study. First, this is a retrospective analysis of specimens taken during endoscopic procedures as part of clinical practice. Typically, real-time analyses of endoscopic procedures provide more realistic information on the utility of endoscopy. Further prospective studies are necessary to assess the utility of the beige mucosa as a predictor or indicator of the histological activity of EoE. Second, a small number of cases of Japanese adult patients were included. In a previous study, beige mucosa was present in 88% of Swedish patients with EoE[2]; therefore, we presume that the beige mucosa can be used for diagnosis of EoE regardless of race. Third, the utility of the beige mucosa with respect to endoscopic outcome indicators, such as response to treatment, is unclear. The relationships of the beige mucosa with tissue findings and potential alterations by therapeutic interventions require additional study.

In conclusion, the observation of beige mucosa under NBI in patients with EoE reflects thinning of the normal differentiated cell layer due to a loss of differentiation of the esophageal squamous epithelium, which is essential to the pathogenesis of EoE. Furthermore, the beige mucosa was considered to be an endoscopic finding that was an indicator of the histological activity of EoE.

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