Introduction

The development in recent years of techniques for endoscopic diagnosis has led to the detection of increasing numbers of cases of early-stage esophageal squamous cell neoplasia.1–4 Although local treatments such as endoscopic resection and local surgical excision are the standards for high-grade intraepithelial neoplasia (HGIN), follow-up is recommended for low-grade intraepithelial neoplasia (LGIN).5,6 Endoscopic diagnosis to differentiate HGIN from LGIN is very important. We previously reported that HGIN turns red within a few minutes after iodine staining (pink-color sign; PCS); however, iodine staining is uncomfortable. By using narrow band imaging (NBI), color change in the area between the intraepithelial papillary capillary loop (background coloration; BGC) is often observed within the brownish area. The diagnostic usefulness of BGC findings for differentiating high-grade intraepithelial neoplasia from low-grade intraepithelial neoplasia was evaluated.

Methods: In a prospective observational study from September 2010 to August 2012, 285 patients who were in a high-risk group for esophageal squamous cell carcinoma underwent endoscopic examination. Lesions with both endoscopic findings of dilated intraepithelial papillary capillary loop on NBI and iodine-unstained areas were studied, in which endoscopic biopsy or endoscopic resection was subsequently performed. The esophageal background mucosa was also evaluated on the basis of the iodine staining pattern (uniform type: Group U, scattered type: Group S).

Results: One hundred three esophageal lesions in 87 patients were studied. When BGC was used as the differentiation index, sensitivity was 93.8%, specificity was 88.2%, and accuracy was 91.3%. When PCS was used, sensitivity was 97.9%, specificity was 88.2%, and accuracy was 93.2% (P = 0.79). In Group U (n = 54), BGC had an accuracy of 93.8%, and PCS had an accuracy of 92.3% (P = 1.0). On the other hand, in Group S (n = 33), BGC had an accuracy of 86.8%, while PCS had an accuracy of 94.7% (P = 0.27).

Conclusions: Diagnosis using BGC on NBI may substitute for diagnosis based on PCS in many patients.

Abstract

Background and Aim: It was previously reported that high-grade intraepithelial neoplasia (HGIN) turns pink within a few minutes after iodine staining (pink-color sign; PCS); however, iodine staining is uncomfortable. By using narrow band imaging (NBI), color change in the area between the intraepithelial papillary capillary loop (background coloration; BGC) is often observed within the brownish area. The diagnostic usefulness of BGC findings for differentiating high-grade intraepithelial neoplasia from low-grade intraepithelial neoplasia was evaluated.

Methods: In a prospective observational study from September 2010 to August 2012, 285 patients who were in a high-risk group for esophageal squamous cell carcinoma underwent endoscopic examination. Lesions with both endoscopic findings of dilated intraepithelial papillary capillary loop on NBI and iodine-unstained areas were studied, in which endoscopic biopsy or endoscopic resection was subsequently performed. The esophageal background mucosa was also evaluated on the basis of the iodine staining pattern (uniform type: Group U, scattered type: Group S).

Results: One hundred three esophageal lesions in 87 patients were studied. When BGC was used as the differentiation index, sensitivity was 93.8%, specificity was 88.2%, and accuracy was 91.3%. When PCS was used, sensitivity was 97.9%, specificity was 88.2%, and accuracy was 93.2% (P = 0.79). In Group U (n = 54), BGC had an accuracy of 93.8%, and PCS had an accuracy of 92.3% (P = 1.0). On the other hand, in Group S (n = 33), BGC had an accuracy of 86.8%, while PCS had an accuracy of 94.7% (P = 0.27).

Conclusions: Diagnosis using BGC on NBI may substitute for diagnosis based on PCS in many patients.

Key words

background coloration, esophageal cancer, esophageal squamous neoplasia, iodine staining, narrow band imaging.

Accepted for publication 12 November 2013.

Correspondence

Dr Masakazu Takahashi, Department of Gastroenterology and Hepatology, Hokkaido University Hospital, Kita 15 jo Nishi 7 chome, Kitaku, Sapporo 060-8638, Japan. Email: mskztakahashi@gmail.com

Conflicts of interest: No financial relationships with a commercial entity producing health-care related products and/or services relevant to this article.
PCS. In this study, we prospectively evaluated the diagnostic usefulness of BGC findings for differentiating HGIN from LGIN of the esophagus and investigated the histopathological mechanism underlying BGC findings.

Methods

Patients. Patients who had previously undergone endoscopic resection for esophageal squamous cell carcinoma (SCC) and patients with current or previous SCC of the head and neck, known to be a high-risk group for metachronous esophageal SCC, were enrolled in this prospective observational study. Patients referred from other hospitals with newly diagnosed esophageal intraepithelial neoplasia because they required more detailed examination were also included (patients of whose biopsy specimen was pathologically reviewed in our institute prior to endoscopic examination were excluded). Patients with prior radiotherapy or chemoradiotherapy for esophageal SCC were excluded. The study subjects underwent endoscopic examination with NBI-ME followed by iodine staining between September 2010 and August 2012 at Hokkaido University Hospital. All lesions showing both endoscopic findings of dilated IPCL on NBI-ME and iodine-unstained areas was classified as scattered type (Fig. 2). Because most carcinomas in situ (HGIN) appearing as unstained lesions are reportedly at least 5 mm in longest diameter, the target lesion in this study was defined as a flat lesion ≥ 5 mm in longest diameter. All patients with target lesions underwent endoscopic biopsy. Patients who were confirmed to have HGIN or SCC by endoscopic biopsy subsequently underwent endoscopic resection or argon plasma coagulation. Lesions showing apparent invasive carcinoma (with irregular surface, depression, or elevation) and reflux esophagitis (linear erosion located near the esophagogastric junction) were diagnosed accordingly and excluded from the study.

In addition, after completion of the study, we conducted an examination on consistencies in the diagnosis of PCS and BGC among doctors. Endoscopic photographs, with a random arrangement of all lesions, were presented. For each lesion, two to four NBI images were presented. Three endoscopists (with endoscopic experience of 25 years, 18 years, and 8 years) conducted the evaluations by selecting BGC-positive or BGC-negative images. In the same manner, with random arrangement of all lesions, two to four endoscopic photographic images showing iodine staining were presented for each lesion. Again, the endoscopists were asked to determine whether the images were positive or negative for PCS.

Histological evaluation. All biopsy materials attached to a filter paper in an extended state were fixed in formalin and then embedded vertically. All resected specimens were cut into longitudinal slices measuring 2 mm in width. The slices were embedded in paraffin and stained with hematoxylin and eosin. All specimens were microscopically reviewed according to World Health Organization (WHO) classification by two pathologists blinded to the clinical characteristics of the patients. In the study on non-neoplastic epithelia remaining in the superficial layers of neoplastic lesions, we measured the three most representative sites in

Figure 1  (a) Endoscopic image with narrow band imaging with magnifying endoscopy (NBI-ME) shows the lesion without color change in the area between the dilated intraepithelial papillary capillary loops. The lesion was diagnosed as background coloration-negative. (b) Endoscopic image with NBI-ME shows the lesion with brownish color change in the area between the dilated intraepithelial papillary capillary loops. The lesion was diagnosed as background coloration-positive.
terms of thickness, and we used the average value thereof (Fig. 3). As for the endoscopic therapy specimens and biopsy specimens containing all mucosal epithelial layers, the thickness of the residual epithelium and that of all mucosal epithelial layers at the same three sites were measured, and average values were calculated in the same manner (Fig. 4).

**Statistical analysis.** Differences in frequency distribution were tested using Fisher’s exact test, and quantitative data were tested using the $t$-test. A $P$ value less than 0.05 was considered to indicate a statistically significant difference. The interobserver agreement was calculated by using the multirater kappa value. The kappa values were interpreted according to Landis and Koch. All analyses were carried out with JMP pro 10 (SAS Institute, Inc., Cary, NC, USA).

**Results**

During the study period, the 285 enrolled patients underwent endoscopic examination with NBI followed by iodine staining. Among them, 87 patients were found to have both endoscopic findings of brownish areas with dilated IPCL on NBI-ME and iodine-unstained areas (103 lesions in total) (Fig. 5). Of the 103 lesions, resected specimens obtained from 42 and biopsy specimens from 61 were examined. The male-to-female ratio was 75:12, and the mean age was 68.7 years (range 49–92 years). The histological diagnosis was SCC/HGIN for 48 lesions and LGIN/non-atypia for 55 lesions (Table 1). When BGC positivity was used as an index of SCC/HGIN, this finding allowed differentiation between SCC/HGIN and LGIN/non-atypia with a sensitivity of 93.8% (95% confidence interval [CI] 90.4–97.1%), specificity of 88.2% (95% CI 85.1–93.1%), and accuracy of 91.3% (95% CI
88.6–93.9%). When PCS was used as the differentiation index, sensitivity was 97.9% (95% CI 95.9–100%), specificity was 88.2% (95% CI 85.1–93.1%), and accuracy was 93.2% (95% CI 90.8–95.6%). The differences between these indices were not significant ($P = 0.79$) (Table 2). Next, differences in diagnostic accuracy for the uniform type (Group U) and the scattered type (Group S) were examined. A diagnosis was made in 54 patients with 65 lesions in Group U (63.1%) and in 33 patients with 38 lesions in Group S (36.9%). The sensitivity, specificity, and accuracy of BGC and PCS were compared between these two groups. In Group U, BGC had a sensitivity of 96.3%, specificity of 92.1%, and accuracy of 93.8%, and PCS had a sensitivity of 96.3%, specificity of 89.5%, and accuracy of 92.3%. Again, there were no significant differences ($P = 1.0$). On the other hand, in Group S, BGC had a sensitivity of 90.5%, specificity of 82.4%, and accuracy of 86.8%, while PCS had a sensitivity of 100%, specificity of 88.2%, and accuracy of 94.7%. BGC had lower values, although the differences did not reach statistical significance ($P = 0.27$) (Table 3).

Interobserver agreement among the three endoscopists for BGC was good, with an estimated kappa value of 0.644 (± 0.042). The agreement for PCS diagnosis was excellent, with an estimated kappa value of 0.827 (± 0.031).

### Table 1 Characteristics of patients and lesions

| Characteristic                      | Value |
|------------------------------------|-------|
| Gender (M/F)                       | 75/12 |
| Age (years old)                    | 68.7 (49–92) |
| Lesion location                     | Upper/middle/lower 10/56/37 |
| Lesion size (mm)                   | Mean 13.0 (5–40) |
| Histological diagnosis             | Non-atypia/LGIN 29/26 |
|                                     | HGIN/LPM 18/25 |
| Specimen                            | Biopsy/EMR, ESD 61/42 |
| Background mucosa                   | Uniform/scattered 65/38 |

EMR, endoscopic mucosal resection; ESD, endoscopic submucosal dissection; HGIN, high-grade intraepithelial neoplasia; LGIN, low-grade intraepithelial neoplasia; LPM, lamina propria mucosae; MM, muscularis mucosae; SM, submucosa.
The histological association between BGC and residual non-neoplastic epithelium thickness was examined. We examined all 42 resected specimens and all 61 biopsy specimens for evaluation of residual non-neoplastic epithelium thicknesses. Subsequently, we examined all 42 resected specimens and 19 of 61 biopsy specimens (confirmed to contain sufficient entire epithelium) for calculation of proportions of the entire thickness of the mucosal epithelium showing neoplasia. Residual non-neoplastic epithelium thicknesses were $118.2 \pm 60.9 \mu m$ in the BGC-negative lesions and $14.3 \pm 18.0 \mu m$ in the BGC-positive lesions, showing a significant difference ($P < 0.01$) (Fig. 6). Three lesions

| BGC | SCC or HGIN | LGIN or non-atypia |
|-----|-------------|--------------------|
| (+) | 45 (93.7%)  | 6 (10.9%)          | 51     |
| (-) | 3 (6.3%)    | 49 (89.1%)         | 52     |
|     | 48          | 55                  | 103    |

Accuracy rate = 91.3%

| PCS | SCC or HGIN | LGIN or non-atypia |
|-----|-------------|--------------------|
| (+) | 47 (97.9%)  | 6 (10.9%)          | 53     |
| (-) | 1 (2.1%)    | 49 (89.1%)         | 50     |
|     | 48          | 55                  | 103    |

Accuracy rate = 91.3%

BGC, background coloration; HGIN, high-grade intraepithelial neoplasia; LGIN, low-grade intraepithelial neoplasia; N.S., not significant; PCS, pink-color sign; SCC, squamous cell carcinoma.

The correlation between histopathological diagnosis and endoscopic diagnosis based on BGC or PCS findings are shown in Tables 2 and 3. The accuracy rates were 91.3% and 92.3% respectively. For the iodine-staining type of background mucosa, the accuracy rates were 93.8% and 92.3% for BGC and PCS, respectively. The results were not statistically significant ($P = 0.79$ and $P = 1.0$).

Table 2 Correlation between histopathological diagnosis and endoscopic diagnosis based on BGC or PCS findings

|       | SCC or HGIN | LGIN or non-atypia |
|-------|-------------|--------------------|
| BGC (+) | 26 (96.3%)  | 3 (7.9%)          | 29     |
| (-)   | 1 (3.7%)    | 35 (92.1%)         | 36     |
|       | 27          | 38                  | 65     |

Accuracy rate = 93.8%

| PCS (+) | SCC or HGIN | LGIN or non-atypia |
|---------|-------------|--------------------|
| (+)     | 21 (100%)   | 2 (11.8%)          | 23     |
| (-)     | 0 (0%)      | 15 (88.2%)         | 15     |
|         | 21          | 17                  | 38     |

Accuracy rate = 94.7%

N.S. ($P = 0.27$)

BGC, background coloration; HGIN, high-grade intraepithelial neoplasia; LGIN, low-grade intraepithelial neoplasia; N.S., not significant; PCS, pink-color sign; SCC, squamous cell carcinoma.

Figure 6  (a) The relationship between the thickness of the non-neoplastic epithelium remaining in the superficial layers of neoplastic lesions and background coloration (BGC): Thickness was lower in the BGC-positive than in the BGC-negative group ($P < 0.01$). (b) The relationship between the proportion of neoplastic cell layers to total epithelial thickness and BGC. The proportion was higher in the BGC-positive than in BGC-negative group ($P < 0.01$).
determined to be BGC-negative were histopathologically diagnosed as SCC/HGIN. In all three lesions, the residual epithelium thickness was 30 μm or less. Two of the three lesions were found in scattered type mucosa and were determined to be PCS-positive by iodine staining. The other lesion showed parakeratosis in the superficial layer of the neoplastic lesion and was also determined to be PCS-negative by iodine staining. Proportions of the entire thickness of the mucosal epithelium showing neoplasia were calculated in 18 BGC-negative lesions and 43 BGC-positive lesions in which the entire mucosal epithelium thickness was measured. The proportions were 0.493 ± 0.190 in the BGC-negative lesions and 0.900 ± 0.118 in the BGC-positive lesions, showing a significant difference (P < 0.01) (Fig. 6).

Discussion

Findings of dilated IPCL by NBI endoscopy are extremely useful for detecting early-stage esophageal squamous cell neoplasia.1,4 Yoshida et al. classified IPCL dilation patterns and reported their usefulness for determining the depth of invasion.17 However, there are very few reports on color changes in areas surrounding IPCL.

According to the WHO classification, morphological features of intraepithelial squamous neoplasia of the esophagus include both architectural and cytological abnormalities, and intraepithelial neoplasia is graded as high-grade when greater abnormalities are detected in the upper half of the epithelium.18 As for the mechanism underlying PCS, HGIN and SCC react only minimally with iodine because of the small number of glycogen-containing cells and are therefore seen as completely unstained areas with a reddish color change after the brown iodine solution fades. On the other hand, LGIN reacts slightly with iodine because of surviving glycogen-containing cells and is therefore seen as an unstained area with a yellowish-white color.9 In this study, examination of BGC also demonstrated residual epithelium thickness to be associated with BGC. Moreover, the thicknesses of the entire mucosal epithelium showing neoplasia essentially corresponded to the HGIN and LGIN criteria defined by WHO. Regarding BGC, Kanzaki et al., who referred to BGC-positive lesions as brownish epithelium, conducted a retrospective study involving mainly patients undergoing HGIN/SCC resection and reported that thinning of the keratinized layer is an important pathogenic factor.19 Our results are consistent with the results of their study. However, the actual mechanism responsible for the color changes remains unknown. The central wavelengths of the NBI filters are 415 and 540 nm, and each has a bandwidth of 30 nm. NBI-ME can clearly visualize the central wavelengths of the NBI filters are 415 and 540 nm, and NBI-ME can clearly visualize the

References

1 Shimizu Y, Takagoshi H, Fujita M, Hosokawa M, Kato M, Asaka M. Endoscopic screening for early esophageal cancer by iodine staining in patients with other current or prior primary cancers. Gastrointest. Endosc. 2001; 53: 1–5.
2 Hashimoto CL, Iriya K, Baba ER et al. Lugol’s dye spray chromoendoscopy establishes early diagnosis of esophageal cancer in patients with primary head and neck cancer. Am. J. Gastroenterol. 2005; 100: 275–82.
3 Takenaka R, Kawahara Y, Okada H et al. Narrow-band imaging provides reliable screening for esophageal malignancy in patients with head and neck cancers. Am. J. Gastroenterol. 2009; 104: 2942–8.
4 Muto M, Minashi K, Yano T et al. Early detection of superficial squamous cell carcinoma in the head and neck region and esophagus by narrow band imaging: a multicenter randomized controlled trial. J. Clin. Oncol. 2010; 28: 1566–72.
5 Gabbert HE, Shimoda T, Hainaut P et al. Squamous cell carcinoma of the oesophagus. In: Hamilton SR, Aaltonen LH, eds. Pathology of human neoplasia. 3rd ed. London: Cambridge University Press, 1994: 411–46.
1. and Genetics of Tumours of the Digestive System: World Health Organization Classification of Tumours. Lyon: IARC Press, 2000; 11–19.

2. Schlemper RJ, Riddell RH, Yamabe H et al. The Vienna classification of gastrointestinal epithelial neoplasia. *Gut* 2000; 47: 251–5.

3. Dixon MF. Gastrointestinal epithelial neoplasia: Vienna revisited. *Gut* 2002; 51: 130–1.

4. Shimizu Y, Kato M, Asaka M et al. Histologic results of EMR for esophageal lesions diagnosed as high-grade intraepithelial squamous neoplasia by endoscopic biopsy. *Gastrointest. Endosc.* 2006; 63: 16–21.

5. Shimizu Y, Omori T, Yokoyama A et al. Endoscopic diagnosis of early squamous neoplasia of the esophagus with iodine staining: high-grade intra-epithelial neoplasia turns pink within a few minutes. *J. Gastroenterol. Hepatol.* 2008; 23: 546–50.

6. Ishihara R, Yamada T, Iishi H et al. Quantitative analysis of the color change after iodine staining for diagnosing esophageal high-grade intraepithelial neoplasia and invasive cancer. *Gastrointest. Endosc.* 2009; 69: 213–18.

7. Ishihara R, Inoue T, Uedo N et al. Significance of each narrow-band imaging finding in diagnosing squamous mucosal high-grade neoplasia of the esophagus. *J. Gastroenterol. Hepatol.* 2010; 25: 1410–15.

8. Shimizu Y, Tukagoshi H, Fujita M, Hosokawa M, Kato M, Asaka M. Metachronous squamous cell carcinoma of esophagus arising after endoscopic mucosal resection. *Gastrointest. Endosc.* 2001; 54: 190–4.

9. Shimizu Y, Tsukagoshi H, Asaka M et al. Head and neck cancer arising after endoscopic mucosal resection for squamous cell carcinoma of the esophagus. *Endoscopy* 2003; 35: 322–6.

10. Ohmori T, Makuuchi H, Kumagai Y. Natural course of iodine unstained area in the esophagus in mass-screening programs. *Stomach Intestine* 1994; 29: 911–19. (in Japanese with English abstract).

11. Yokoyama A, Ohmori T, Takahashi H et al. Successful screening for early esophageal cancer in alcoholics using endoscopy and mucosal iodine staining. *Cancer* 1995; 76: 928–34.

12. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977; 33: 159–74.

13. Yoshida T, Inoue H, Usui S, Satodate H, Fukami N, Kudo SE. Narrow-band imaging system with magnifying endoscopy for superficial esophageal lesions. *Gastrointest. Endosc.* 2004; 59: 288–95.

14. Kanzaki H, Ishihara R, Ishiguro S et al. Histological features responsible for brownish epithelium in squamous neoplasia of the esophagus by narrow band imaging. *J. Gastroenterol. Hepatol.* 2013; 28: 274–8.

15. Gono K, Obi T, Yamaguchi M et al. Appearance of enhanced tissue features in narrow-band endoscopic imaging. *J. Biomed. Opt.* 2004; 9: 568–77.

16. Muto M, Hironaka S, Nakane M, Boku N, Ohtsu A, Yoshida S. Association of multiple Lugol-voiding lesions with synchronous and metachronous esophageal squamous cell carcinoma in patients with head and neck cancer. *Gastrointest. Endosc.* 2002; 56: 517–21.