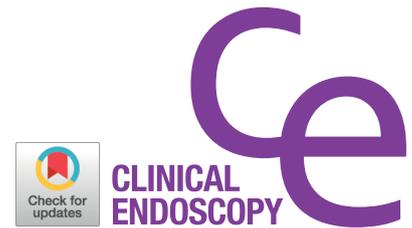


## CASE REPORT

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# New Diagnostic Approach for Esophageal Squamous Cell Neoplasms Using Linked Color Imaging and Blue Laser Imaging Combined with Iodine Staining

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A 62-year-old man with a flat early esophageal cancer was referred for endoscopic treatment. White light imaging revealed a pale red lesion, whereas linked color imaging (LCI) and blue laser imaging (BLI) yielded purple and brown images, respectively. Iodine staining demonstrated a large unstained area with a homogenous but very weak pink-color sign. This area appeared more clearly as purple and green on LCI and BLI, respectively; however, a different colored portion was observed at the 4 o'clock position inside the iodine-unstained area. Histopathology findings of the resected specimen revealed squamous intraepithelial neoplasia at the 4 o'clock position and an esophageal squamous cell carcinoma in the remaining iodine-unstained area. LCI and BLI combined with iodine staining produce characteristic images that overcome the pink-color sign, reflecting the histological features of a flat esophageal neoplasm. This new method is useful for detailed evaluation of early flat squamous cell neoplasms. **Clin Endosc** 2019;52:497-501

**Key Words:** Early esophageal cancer; Image enhanced endoscopy; Linked color imaging; Blue laser imaging; Iodine staining

## INTRODUCTION

Endoscopy enables diagnosis of neoplastic lesions at an early stage, which may be amenable to endoscopic treatment to preserve the organ.<sup>1</sup> Early esophageal neoplasms pose a diagnostic challenge using white light imaging (WLI) and image-enhanced endoscopy including narrow band imaging (NBI)<sup>2-5</sup> and blue laser imaging (BLI).<sup>6-8</sup> WLI combined with iodine staining has been routinely performed as unstained areas are visualized as a pink-color sign in presence of malignancy.<sup>9</sup> Occasionally, differentiation of malignant lesions to

be treated from other unstained lesions is difficult using these diagnostic modalities.<sup>10</sup>

The current study attempted to demonstrate the lesion in a patient with an early flat esophageal neoplasm using linked color imaging (LCI) and BLI combined with iodine staining. We present a new finding that LCI and BLI combined with iodine staining can produce characteristic images that overcome the pink-color sign and reflect in detail the histological features of a flat esophageal neoplasm.

## CASE REPORT

A 62-year-old man with a flat type early esophageal cancer was referred for endoscopic submucosal dissection. Hypopharyngeal carcinoma was synchronously diagnosed by using upper gastrointestinal endoscopy by the referring physician. History of the patient revealed a habit of smoking (10 cigarettes/day for 42 years) and drinking alcohol (700 mL beer/day). Physical examination revealed a mass in the right

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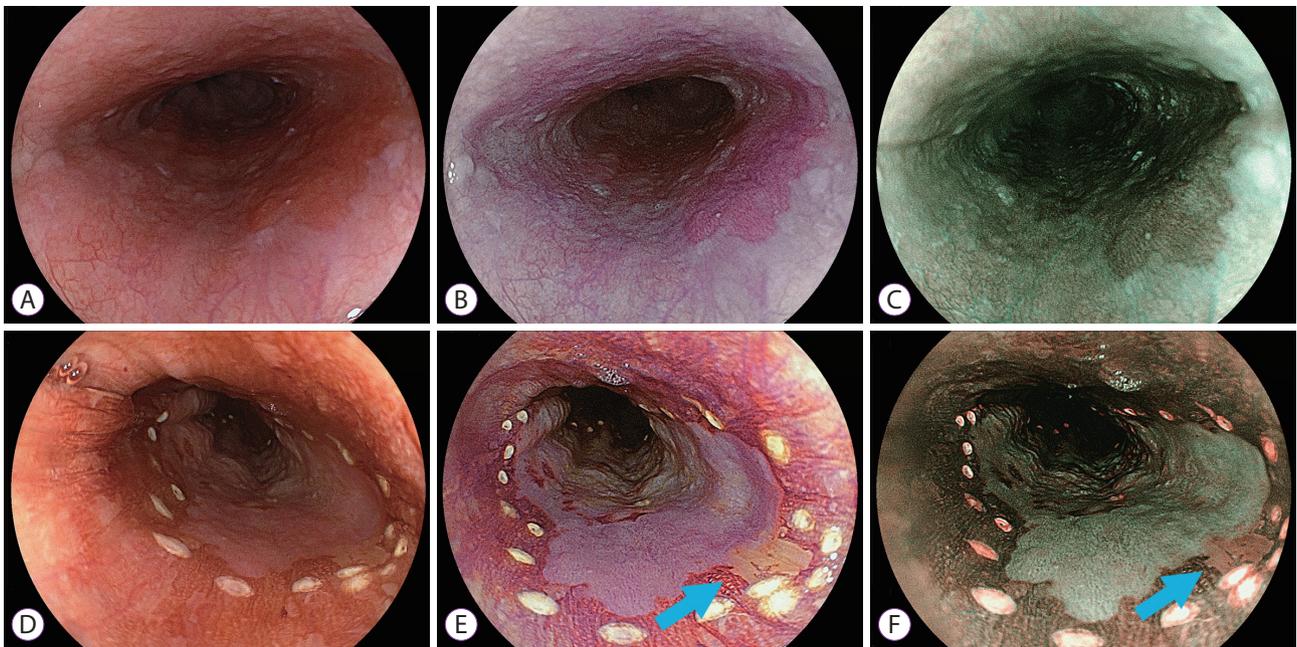
neck caused by the hypopharyngeal carcinoma and no other abnormal findings. Baseline laboratory investigations were within normal limits. A computed tomography scan from the neck to the pelvis showed a tumor in the right hypopharynx, but no metastatic lesions.

A 50-mm superficial esophageal cancer (0-IIb) was revealed in the distal esophagus using a laser endoscopic system (EG-L590ZW, LL-7000 and VP-7000; Fujifilm Co., Tokyo, Japan). WLI revealed a pale red lesion, seen as purple on LCI and brown on BLI (Fig. 1). Magnified BLI revealed abnormal intrapapillary capillary loops (type B1).<sup>11</sup> After staining with iodine solution, a large iodine-unstained area with an irregular shape was seen and the pink-color sign was positive but very weak under WLI. These areas remained purple on LCI and changed to green on BLI. The important issue is that the entire lesion was uniformly positive for the pink-color sign on WLI, whereas interestingly, a pale yellow area on LCI and brown area on BLI were found at the 4 o'clock position inside the iodine-unstained lesion, suggesting different histological findings (Fig. 1). These areas were evaluated by calculating the difference in color using the method of the Commission Internationale de l'Eclairage (CIE), as reported previously<sup>12</sup> and by comparing with the histological findings of the resected specimens. Using this method, the difference was expressed as the distance between two locations corresponding to two colors in the CIE color space, which define a three-dimensional

space ( $L^*$ ,  $a^*$  and  $b^*$ ) as a sphere. Greater distance implies high contrast with a high color difference. This was performed using commercially available image analysis software (Adobe Photoshop; Adobe Systems, San Jose, CA, USA). The color difference between the two regions was 28.9 on LCI and 26.6 on BLI both of which were higher than the 5.8 observed on WLI. Subsequently, markings were placed 2 or 3 mm outside the demarcated area and the lesion was resected using endoscopic submucosal dissection. Histopathologic findings of the resected specimen did not show esophageal squamous cell carcinoma but rather squamous intraepithelial neoplasia at the 4 o'clock position, and showed squamous cell carcinoma in the remaining iodine-unstained area (Fig. 2).

## DISCUSSION

In this patient, LCI and BLI combined with iodine staining produced characteristic images that eliminated the pink-color sign seen on WLI and reflected the histological features of the flat esophageal neoplasm. Purple and green mucosa after iodine staining was readily identifiable, compared to the pink-color sign. In addition, this new modality could differentiate squamous cell carcinoma from squamous intraepithelial neoplasm by the difference in color. To the best of our knowledge, this is the first report of endoscopic findings using LCI

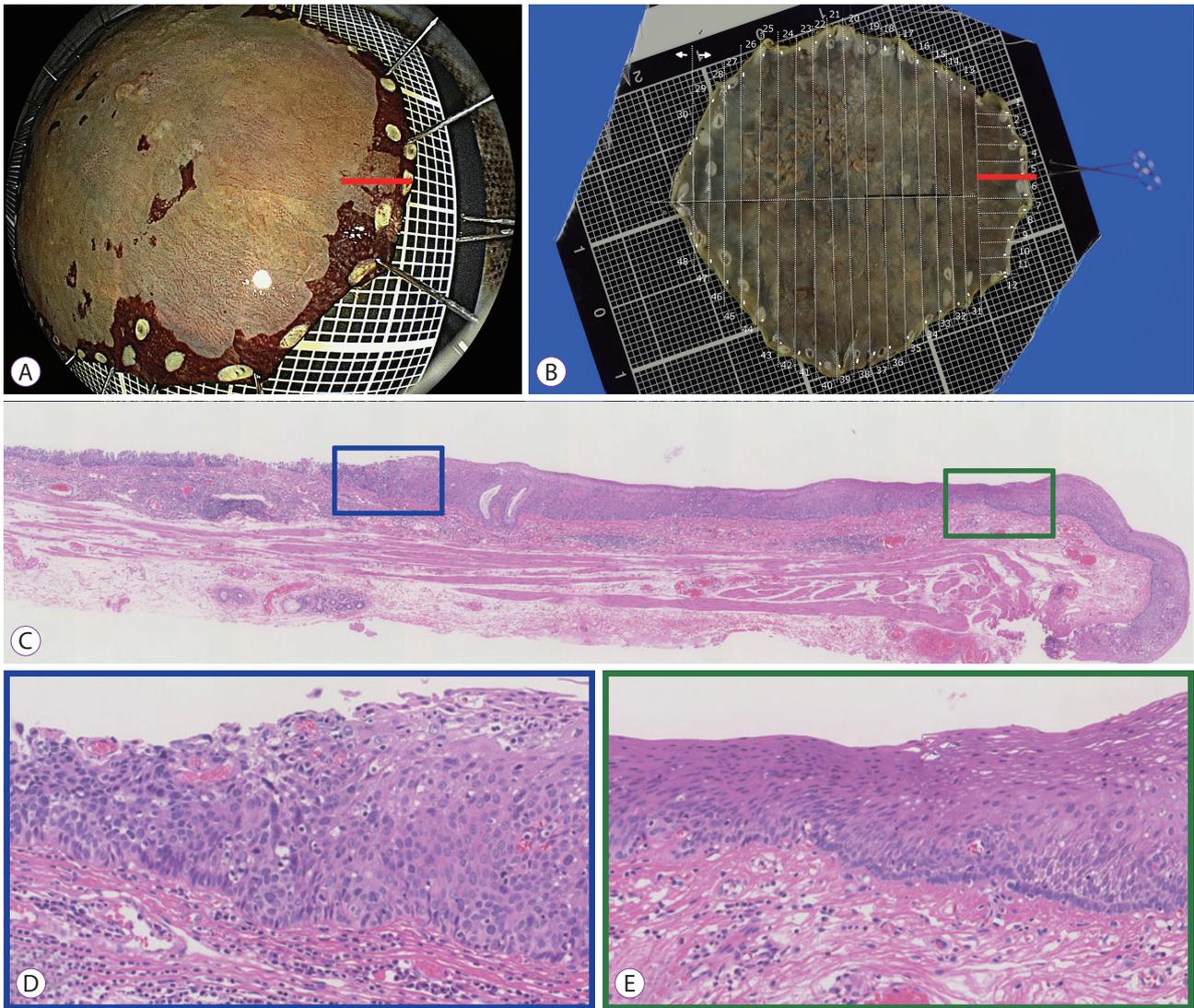


**Fig. 1.** Endoscopic images of esophageal squamous cell neoplasm. (A-C) Before iodine staining. (A) White light imaging shows slightly red lesion from the 3 to 5 o'clock position. (B) Linked color imaging shows a purple lesion. (C) Blue laser imaging shows a brown lesion. (D-F) Three minutes 35 seconds after iodine staining. (D) The entire lesion has a positive pink-color sign on white light imaging. (E) Linked color imaging shows a purple area in most of the main lesion but pale yellow mucosa at the 4 o'clock position (light blue arrow). (F) Blue laser imaging shows a green lesion in most of the main lesion but there is brown mucosa at the 4 o'clock position (light blue arrow).

and BLI that provides new insights into the accurate diagnosis of esophageal squamous cell carcinoma.

Several endoscopic modalities have been developed to

screen for early esophageal cancers such as WLI combined with iodine staining and image enhanced endoscopy. The prevalence of esophageal cancer is increasing worldwide.



**Fig. 2.** Histological features of the resected specimen. (A, B) Endoscopically resected specimen—Red line indicates the area with different colors on linked color imaging and blue laser imaging. (C) Histopathological findings of the lesion indicated by the red line on image A and B (hematoxylin & eosin,  $\times 2.57$ ). (D) A magnified image shows the histopathological findings at the border between squamous cell carcinoma (left half) and intraepithelial neoplasia (right half) (hematoxylin & eosin,  $\times 8.17$ ). (E) The magnified image shows the histopathological findings at the border between intraepithelial neoplasia (left half) and normal mucosa (right half) (hematoxylin & eosin,  $\times 8.17$ ).

**Table 1.** Color Differences between the Malignant Region and Dysplastic Area (Calculated by the Commission Internationale de l’Eclairage Method)

After iodine staining	$\Delta E$ (Cancer and dysplastic regions color difference)	$\Delta E$ (Cancer and surrounding epithelium color difference)
WLI	5.8	14.0
BLI	26.6	19.7
LCI	28.9	21.3

By National Bureau of Standards unit the color difference evaluation: 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 and higher Very much.

BLI, blue laser imaging; LCI, linked color imaging; WLI, white light imaging.

Therefore, appropriate knowledge of the utilization of these modalities is essential, facilitating the early detection of cancer as well as minimally invasive endoscopic treatment, in order to improve the quality of life of patients and reduce the mortality associated with esophageal squamous cell carcinoma.

Detection of superficial flat type esophageal squamous cell carcinoma is often difficult as only subtle changes are revealed in the mucosal surface. An accurate examination with iodine staining includes assessment of the pink-color sign, wherein color change is observed from an unstained area to a pink color three minutes after application of the iodine solution. The iodine solution adheres to the glycogen of the normal non-keratinized squamous epithelium of the esophagus, whereas a malignant tissue, dysplasia site, inflammatory site, or erosive mucosa lacks glycogen and remains unstained.<sup>13</sup> However, accurate judgment of positive or negative pink-color sign may not be done by endoscopists owing to minimal difference between the pale pink stained area and non-pink color of the unstained area. In this patient, WLI demonstrated a pale pink color in the unstained area, which may have been difficult to judge as positive or negative.

Recently, image enhanced endoscopy was developed and the ability of this technique to detect esophageal lesions was reported to be greater than WLI, regardless of iodine staining.<sup>14</sup> Esophageal squamous cell carcinoma appears as a silver metallic color on NBI after staining with iodine.<sup>15</sup> However, multiple lesions or irregularly shaped areas may be visualized in iodine-unstained areas, thereby causing difficulty in recognition and precise ability to distinguish the cancer.<sup>10</sup> In the present patient, BLI revealed green mucosa in the unstained area and interestingly LCI showed purple mucosa, both of which allowed the endoscopist to identify an abnormal lesion compared with the use of WLI. Visualization of partially non-purple and non-green portions in the unstained area using LCI and BLI, respectively was unanticipated. Endoscopic submucosal dissection specimens revealed that the purple and green areas were squamous cell carcinoma whereas the non-purple and non-green areas were squamous intraepithelial neoplasia. These findings may be useful to identify squamous cell carcinoma among multiple iodine-unstained lesions that can often be observed in patients with risk factors such as smoking and intake of alcohol.

Previous studies have reported the usefulness of color differences in the identification of malignant lesions.<sup>16,17</sup> In the present case; the color difference was calculated in all three modalities including WLI, LCI, and BLI before and after iodine staining and comparison of the malignant lesion was performed with the surrounding mucosa as well as the dysplastic regions (Table 1). These results showed that the color differences between esophageal squamous cell carcinoma

and dysplasia as well as those between esophageal squamous cell carcinoma and normal mucosa were greatest using LCI, which explains the easier recognition by visual inspection. The reason for greater color difference on LCI remains unclear; however, it may be hypothesized that the presence or absence of normal squamous cells in the epithelium may influence the color visualized. Malignant cells are evident in the entire layer of esophageal epithelium in carcinoma but not in areas of intraepithelial neoplasia that include normal squamous cells in the superficial layer. LCI and BLI have high emission intensity of short wavelengths that reflect from the mucosal surface.<sup>18</sup> This histological difference may influence the spectral reflectance of both epithelia even after iodine staining, which results in different colors.

In conclusion, LCI and BLI combined with iodine staining produced characteristic images that overcame the pink-color sign, reflecting histological features of a flat esophageal neoplasm. This new approach is useful to evaluate and diagnose early flat squamous cell neoplasms in detail, and deserves further study.

#### Conflicts of Interest

Hiroyuki Osawa and Hironori Yamamoto received honoraria from Fuji-film Corporation and Fujifilm Medical Corporation. Other authors have no conflicts of interest.

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