Endoscopic diagnosis of early squamous neoplasia of the esophagus with iodine staining: High-grade intra-epithelial neoplasia turns pink within a few minutes

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Abstract

Background and Aim: The ability to detect early squamous neoplasia of the esophagus can be enhanced considerably by iodine staining during endoscopic examination; however, there has been no study on distinguishing high-grade intra-epithelial squamous neoplasia from low-grade dysplasia by endoscopic examination. We assumed that high-grade intra-epithelial neoplasia could be identified as iodine-unstained areas more distinct and reddish than low-grade dysplasia after the brown color of iodine solution has faded, because there is almost no remaining glycogen-containing epithelium in high-grade intra-epithelial neoplasia.

Methods: Seventy-nine patients who were found to have demarcated iodine-unstained areas (0.5 cm to 1.5 cm at widest part, 121 lesions in total) were studied. After a target lesion was found, the lesion was observed for about 3 min and its discoloration was evaluated. If a light-pink part appeared in the iodine-unstained area, the lesion was regarded as positive for pink color. If no light-pink part was observed in the lesion within 3 min, the lesion was regarded as negative for pink color.

Results: Thirty-four (87.2%) of the 39 lesions diagnosed as pink-color positive were histologically confirmed to be high-grade intra-epithelial squamous neoplasia or squamous cell carcinoma, whereas only three (3.7%) of the 82 lesions diagnosed as negative for pink color were histologically confirmed to be high-grade intra-epithelial squamous neoplasia (P < 0.0001). Using the pink-color sign as a diagnostic index for high-grade intra-epithelial neoplasia and squamous cell carcinoma, sensitivity was 91.9% and specificity was 94.0%.

Conclusion: By using the pink-color sign for endoscopic diagnosis, accurate diagnosis without endoscopic biopsy for iodine-unstained areas was possible.
According to WHO classification, intra-epithelial neoplasia is graded as high-grade when greater abnormalities occur in the upper half of the epithelium.\(^\text{10}\) Iodine-unstained areas are associated with lesser amounts of glycogen in the squamous cell epithelium than are stained areas.\(^\text{8,14,15}\) We assumed that high-grade intra-epithelial squamous neoplasia could be identified as more distinct iodine-unstained areas than low-grade dysplasia because there is almost no remaining normal epithelium which contain glycogen. The purpose of this study was to determine the actual findings of high-grade intra-epithelial squamous neoplasia and squamous cell carcinoma under endoscopic iodine staining.

**Methods**

Since most carcinomas in situ (high-grade intra-epithelial squamous neoplasia) appearing as unstained lesions have been reported to be 5 mm or more at the widest part,\(^\text{16,17}\) and since we previously reported that a flat-type lesion less than 1 cm at the widest part might be regarded as intra-epithelial neoplasia,\(^\text{13}\) the target lesion in this study was defined as a flat, iodine-unstained area of about 1 cm (0.5 cm to 1.5 cm at the widest part). After a target lesion was found, the lesion was observed for about 3 min (until the brown color of iodine solution smeared on the mucosal surface had faded), and its discoloration was evaluated. If a light-pink part appeared in the iodine-unstained area, the lesion was regarded as being positive for pink color. If no light-pink part was observed in the lesion within 3 min, the lesion was regarded as being negative for pink color.

During the period from March 2002 to December 2005, 530 patients underwent gastrointestinal endoscopy with iodine staining by our study group at Hokkaido University Hospital. Seventy-nine of those patients were found to have demarcated iodine-unstained areas (121 lesions in total) and underwent endoscopic biopsy after evaluation of the pink-color sign. Lesions diagnosed as apparent invasive carcinoma (with irregular surface, depression or elevation) and reflux esophagitis (linear erosion located near the esophagogastric junction) were excluded from this study. Our study group included three experienced endoscopists (YS, JY, YO). We made final endoscopic diagnosis of pink-color sign for each case after discussion. The patients included 67 men and 12 women with a mean age (±SD) of 62.2 ± 8.7 years. In 52 of those patients, the lesions were detected by endoscopic screening with iodine staining for high-risk populations, and the lesions were detected in the other 27 patients by endoscopic follow up after EMR of esophageal carcinoma (Table 1). Patients who were confirmed as having high-grade intra-epithelial neoplasia or invasive squamous cell carcinoma by endoscopic biopsy subsequently underwent EMR. Endoscopic examinations were performed with videoendoscopes (XQ-230, Q-240; Olympus, Tokyo, Japan. EG-410 HR; Fujinon-Toshiba, Saitama, Japan), and iodine-staining methods were performed with 1.5% iodine solution.

All biopsy specimens were cut in the center, and all resected specimens were cut into longitudinal slices measuring 2 mm in width. The slices were embedded in paraffin and stained with hematoxylin–eosin. All specimens were microscopically reviewed by two pathologists blinded to the clinical characteristics of the patients.

The study was approved by the ethics committee of our institute, and written informed consent for all procedures and for participation in this study was obtained from all subjects.

Differences in frequency distribution were tested using the χ² test. A P-value less than 0.05 was considered to indicate statistical significance.

**Results**

Histological examination of biopsy specimens revealed that 32 of the 79 patients had high-grade intra-epithelial neoplasia or invasive squamous cell carcinoma. All of the 32 patients underwent EMR at Hokkaido University Hospital. Totally resected specimens from the 32 patients (37 lesions) were used for histological examination. In the remaining 47 patients (84 lesions), biopsy specimens were used for histological examination. Complications due to EMR (such as hemorrhage and perforation) did not arise in any of the patients.

Of the 121 demarcated iodine-unstained areas (79 patients) that were studied, 39 lesions (31 patients) were endoscopically diagnosed as positive for pink color and 82 lesions (48 patients) were endoscopically diagnosed as negative for pink color before endoscopic biopsy. Among the 39 lesions diagnosed as positive for pink color, 29 lesions were histologically confirmed to be high-grade intra-epithelial neoplasia, five lesions were confirmed to be squamous cell carcinoma with invasion of lamina propria mucosae and four lesions were confirmed to be inflammation. Among the 82 lesions diagnosed as negative for pink color, three lesions were histologically confirmed to be high-grade intra-epithelial neoplasia, 57 lesions were confirmed to be low-grade dysplasia and 22 lesions were confirmed to be inflammation or epithelial atrophy (Table 2). Thirty-four (87.2%) of the 39 lesions diagnosed as positive for pink color were high-grade intra-epithelial neoplasia or squamous cell carcinoma, whereas only three (3.7%) of the 82 lesions diagnosed as negative for pink color were high-grade intra-epithelial neoplasia (P < 0.0001). Using the pink-color sign as a diagnostic index for high-grade intra-epithelial neoplasia and squamous cell carcinoma, sensitivity was 91.9% and specificity was 94.0%.

A case of a pink-color-sign lesion in a 65-year-old-man histologically confirmed to be high-grade intra-epithelial squamous

| Table 1 | Reasons for upper endoscopic examination in the 79 patients who were found to have demarcated iodine-unstained areas and underwent endoscopic biopsy |
|---------|---------------------------------------------------------------------------------
| Alcohol and tobacco abuse \(^1\) | 13 |
| Other primary cancers, head and neck cancer | 27 |
| Lung cancer | 2 |
| Gastric cancer | 7 |
| Colon cancer | 3 |
| Follow up after EMR of esophageal cancer | 27 |

\(^1\) Men over the age of 50 with a history of tobacco abuse (defined as more than 20 pack-years) and/or alcohol abuse (more than five days of alcohol consumption per week). All were drinkers, and 11 were both drinkers and smokers.
neoplasia is shown in Fig. 1. A case of a pink-color-sign lesion and a lesion negative for pink color in a 64-year-old-man histologically confirmed to be invasive squamous cell carcinoma and low-grade dysplasia, respectively, is shown in Fig. 2.

**Discussion**

The iodine-staining method was first established by Schiller for early diagnosis of uterine cervix neoplasia and was developed in gynecologic colposcopy. In the English-language literature concerning the esophagus, Brodmerkel first presented results of iodine staining performed during endoscopic examination and proposed its usefulness in the diagnosis of squamous cell carcinoma of the esophagus in 1971. Although various techniques for endoscopic diagnosis have been developed over the past three decades, iodine staining is still the most useful method for screening of early esophageal carcinoma. The mechanism of iodine staining of the squamous epithelium is considered to be as follows: glycogen produced in the normal squamous cell epithelium interacts with iodine and shows a brown color, and the areas with lesser amounts of glycogen in the squamous cell epithelium (because of a defect of epithelium or abnormal epithelium) hardly interact with iodine. For that reason, squamous neoplasia and esophagitis are shown as iodine-unstained areas. As for the distribution of glycogen-containing cells in the squamous epithelium of the esophagus, in vivo and in vitro vital staining using periodic acid Schiff (PAS) and PAS-diastase staining methods have revealed that more glycogen-containing cells exist in the middle-to-deep cell layer than in the surface cell layer of epithelium. Therefore, a lesion in which the lower half or more of the epithelium is occupied by abnormal cells would hardly react with iodine.

According to the WHO classification, morphological features of intra-epithelial squamous neoplasia of the esophagus include both architectural and cytological abnormalities, and intra-epithelial neoplasia is graded as high-grade when greater abnormalities occur in the upper half of the epithelium. High-grade intra-epithelial neoplasia and squamous cell carcinoma hardly react with iodine due to the small number of glycogen-containing cells and are therefore seen as completely unstained areas with a reddish color after the brown color of iodine solution has faded. On the other hand, low-grade dysplasia reacts slightly with iodine due to

<table>
<thead>
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<th>Table 2</th>
<th>Histological diagnosis for 121 demarcated iodine-unstained areas that were endoscopically diagnosed as pink-color positive or pink-color negative</th>
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<tr>
<td></td>
<td>Pink-color sign</td>
</tr>
<tr>
<td>39 lesions</td>
<td>82 lesions</td>
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<tr>
<td>(31 cases)</td>
<td>(48 cases)</td>
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<tr>
<td>Invasive squamous cell carcinoma</td>
<td>5 0</td>
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<tr>
<td>High-grade intra-epithelial neoplasia</td>
<td>29 3</td>
</tr>
<tr>
<td>Low-grade dysplasia</td>
<td>1 57</td>
</tr>
<tr>
<td>Inflammation or epithelial atrophy</td>
<td>4 22</td>
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P < 0.0001.

*Squamous cell carcinoma invaded to lamina propria mucosae.
remaining glycogen-containing cells and is therefore seen as an unstained area with a yellowish-white color.

During endoscopic examination with iodine staining, the pattern of iodine-unstained areas varies. Muto et al. reported that patients with head and neck cancer, who have a very high risk for squamous cell carcinoma of the esophagus, were frequently associated with multiple iodine unstained areas, and that 55% of such patients had synchronous second primary squamous cell carcinomas of the esophagus.20 We have also reported squamous cell carcinoma of the esophagus is often associated with background mucosa showing many minute, non-cancerous unstained areas (scattered type).7 We found that metachronous multiple esophageal carcinomas after initial EMR arose more frequently in patients with scattered-type background mucosa than in patients with uniformly iodine-stained background mucosa (uniform type).7 Periodic endoscopic examination with iodine staining is therefore especially important for patients with esophageal mucosa showing multiple iodine-unstained areas. It has been difficult to distinguish high-grade intra-epithelial neoplasia from low-grade dysplasia without histological diagnosis of endoscopic biopsy specimens. Multiple endoscopic biopsies have been required for diagnosis of patients with esophageal mucosa showing multiple iodine-unstained areas. Multiple endoscopic biopsies take effort and are time consuming, endoscopic biopsy also causes mucosal oozing and consequently makes it difficult to find other target lesions. We believe that endoscopic diagnosis using the pink-color sign enables accurate diagnosis without endoscopic biopsy.

Figure 2  (a) Endoscopic image with iodine staining shows multiple small iodine-unstained areas in the lower esophagus (scattered type) in a 64-year-old man. (b) Endoscopic image obtained about 3 min after iodine staining shows a demarcated reddish lesion (arrow 1) and other unstained areas with yellowish-white color. The lesion indicated by arrow 1 was diagnosed as pink-color positive and other unstained areas were diagnosed as pink-color negative. (c) Resected specimen of the lesion indicated by arrow 1 was histologically diagnosed as squamous cell carcinoma slightly invading the lamina propria mucosae (hematoxylin–eosin, original magnification x100). (d) Biopsy specimen obtained from the lesion indicated by arrow 2 in part b was histologically diagnosed as low-grade dysplasia (hematoxylin–eosin, original magnification x400).
Even if the pink-color sign is used, endoscopic diagnosis for high-grade intra-epithelial neoplasia and mucosal carcinoma is still difficult. Although endoscopic ultrasonography is useful for distinguishing between mucosal carcinoma and submucosal carcinoma, accurate diagnosis of mucosal carcinoma and intra-epithelial neoplasia by diagnostic imaging is difficult. We previously reported that over 30% of esophageal lesions diagnosed by endoscopic biopsy as high-grade intra-epithelial neoplasia of the esophagus revealed to be invasive carcinoma by EMR specimens, and we suggested that high-grade intra-epithelial neoplasia of the esophagus has characteristics of carcinoma in the preinvasive stage. The treatment strategy for patients with high-grade intra-epithelial neoplasia of the esophagus should be the same as that for patients with mucosal carcinoma.

As for other novel diagnostic modalities, narrow-band imaging (NBI) has recently been developed as a new method for detecting early neoplasia of the gastrointestinal tract. Momma et al. reported the usefulness of NBI for detecting early squamous cell carcinoma of the esophagus without iodine staining; however, there has been no study on the usefulness of NBI for detecting intra-epithelial squamous neoplasia including low-grade dysplasia. Further study comparing pink-color sign on iodine staining with NBI is required.

Although diagnosis of iodine-unstained areas with the pink-color sign seems to prolong examination time for a few minutes, unnecessary endoscopic biopsies can be avoided by using the pink-color sign and the use of this method will enable not only accurate endoscopic diagnosis but also safer endoscopic examination and shortened total examination time.

References
