Videoendoscopy with vital double dye staining (crystal violet and methylene blue) for detection of a minute focus of early stage adenocarcinoma in Barrett’s esophagus: a case report

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Barrett’s esophagus is a condition in which the normal stratified squamous esophageal epithelium is replaced by metaplastic columnar epithelium.\(^1\) Intestinal metaplasia leads to an increased risk for adenocarcinoma\(^2\); the incidence of adenocarcinoma associated with Barrett’s esophagus has increased over the last decade.\(^3\) Traditionally the preferred curative treatment has been esophagectomy. However, the management of early-stage adenocarcinoma in Barrett’s epithelium is changing. Initial reports have appeared concerning promising results with photodynamic therapy\(^6\) and argon plasma coagulation.\(^7\) Ell et al.\(^3\) reported successful treatment of early-stage esophageal cancer in a large number of patients with Barrett’s esophagus by using endoscopic mucosal resection (EMR). Although previously considered hazardous, the safety and efficacy of this type of endoscopic treatment have improved. In contrast to ablative treatments, EMR provides a specimen for histologic assessment.\(^3,8\) However, because there is a risk of recurrence even after apparent cure, correct endoscopic diagnosis and assessment of adjacent mucosa is essential. This is a description of successful endoscopic diagnosis and treatment of an extremely small, flat esophageal adenocarcinoma in a patient with Barrett’s esophagus by using a two-channel high-resolution videoendoscope and sequential double dye staining followed by EMR.

**CASE REPORT**

A 78-year-old man presented with heartburn and nausea. A diagnosis had been made 5 years earlier of reflux esophagitis associated with short segment Barrett’s esophagus. Examination showed no enlargement of superficial lymph nodes. Laboratory tests disclosed a hemoglobin of 11.0 gm/dL (14-18 gm/dL).

Five minutes before endoscopy, the patient ingested 40 mg of dimethyl polysiloxane (DMPS) (Gascon; Kissei Pharmaceutical Co., Ltd., Matsumoto, Japan) and 20,000 U of pronase (Pronase MS; Kaken Pharmaceutical Co., Ltd., Tokyo, Japan) with 50 mL of warm water. Upper endoscopy with a high-resolution videoendoscope (EG 410HRT; Fuji Photo Co., Ltd., Omiya, Japan) disclosed a 3-cm segment of columnar-appearing epithelium. Mucus was removed from the surface by washing with 100 mL of water. No lesion was evident before staining (Fig. 1A). However, after spray application of 0.1% methylene blue solution,\(^13\)\(^15\) a slightly pink, unstained area in contrast to the stained metaplastic mucosa was identified (Fig. 1B). Because of a suspicion that the lesion was dysplastic, a forceps biopsy specimen was obtained. Histologic examination showed adenocarcinoma. Clinical staging was performed based on endoscopy, EUS, abdominal US, and CT of the chest and abdomen. Unfortunately, EUS with a 20-MHz catheter probe (VSP-701, Fujinon, Tokyo, Japan) did not clearly detect the small lesion. The lesion was diagnosed endoscopically as type 0-IIb (flat type) based on the generally accepted Japanese criteria for local treatment of early esophageal squamous cell carcinoma.\(^16\) The tumor was judged to be confined to the mucosa (T1s or T1a).\(^16\) No regional or distant metastases were found.

At endoscopy 1 month later with a high-resolution two-channel videoendoscope (EG410Dcw; Fuji Photo) the cancer could not be detected before staining. Three minutes
after spray application of 20 mL of 0.1% methylene blue, sharp contrast was noted between the unstained lesion and the blue-stained metaplastic Barrett’s epithelium (Fig. 2A). Next, 1 mL of 0.05% crystal violet solution was sprayed and within 1 minute the previously unstained lesion was stained by the crystal violet. A mucosal pattern indicative of cancer (Fig. 2B) was delineated in which the glands varied in size and shape. The size of the flat lesion was estimated at 3 × 3 mm. EMR was performed with a strip biopsy technique. Initially 1 mL of 10% glucose with 0.005% epinephrine was injected into the submucosa beneath the lesion. Then the lesion was pulled upwards, encircled with a snare, and rapidly resected with a mixed-phase high-frequency electrosurgical device. The tumor was removed completely (Fig. 2C).

Histopathologic assessment of the resected specimen disclosed early-stage adenocarcinoma (Fig. 2D) according to criteria of the World Health Organization. No complications including perforation or significant bleeding occurred. The patient was permitted to eat a light meal after 12 hours and was discharged after 2 days. Endoscopy 5 months later showed no cancer, and biopsy specimens obtained from the resection site no residual tumor.

DISCUSSION

There has been a gradual increase in the number of mucosal adenocarcinomas of the esophagus detected by endoscopy. As illustrated by the present case, chromoendoscopy is the most reliable method for identifying small flat or slightly depressed lesions. Canto et al. reported that methylene blue accurately stains areas of intestinal metaplasia in Barrett’s esophagus. This dye appears to be absorbed through the cell membrane into the cytoplasm, but details of the mechanism are unknown. In our patient, application of methylene blue showed the cancer as a paucity of blue staining that differed from surrounding deeply stained metaplastic epithelium. Epithelial cancer evidently may lack the dye-absorbing properties of nonneoplastic epithelium at sites of intestinal metaplasia. However, whether chromoendoscopy with methylene blue can detect dysplasia and cancer within areas of intestinal metaplasia remains controversial.

After endoscopic observation of methylene blue staining, crystal violet was applied and this stained the irregularly contoured mucosal glands of the cancer in our patient; crystal violet is known to stain both mucosa with intestinal metaplasia and cancer. This dye was originally used at gastroscopy for measuring the pH of the gastric mucosal surface. The pharmacologic actions of crystal violet have not...
been studied extensively and thus remain poorly understood. It is best known as a topical antimicrobial agent that irreversibly binds microbial DNA and directly inhibits cell replication.\textsuperscript{19} Crystal violet stainsthe nuclei of normal and cancerous cells,\textsuperscript{17} making it useful for morphologic analysis of the sur-
face structure of minute cancers. The use of crystal violet after methylene blue staining and a high-res-
olution videoendoscope showed the detailed mucosal structure of this early stage adenocarcinoma aris-
ing in Barrett’s esophagus.

Premedication with DMPS and pronase improves visualization during chromoendoscopy.\textsuperscript{20} The most important requirement for achieving a finely delineated dye-staining pattern is removal of mucus from the lesion with water. The glands of the lesion described here were irregular in shape and varied in size; this appearance is similar to that of early-stage colon carci-
noma designated “p5a; amorphous appearance” in Japan.\textsuperscript{21-23} As in our previous study of 28 patients with
Barrett’s or non-Barrett’s esophagus, no side effects of staining were observed in the present case.\textsuperscript{24}

Various endoscopic methods have been used to treat early stage cancer in Barrett’s esophagus as alternatives to traditional surgical resection. These procedures include endoscopic mucosectomy,\textsuperscript{3} as well as ablative methods such as photodynamic therapy,\textsuperscript{25} laser therapy,\textsuperscript{26} and argon plasma coagulation.\textsuperscript{7} EMR is being used increasingly for treatment of superfical esophageal squamous cell carcinoma in Japan.\textsuperscript{9,10,12} A major advantage of EMR over ablative methods is that the resected specimen can be examined histologically. Various EMR techniques are available.\textsuperscript{9,10,12} To permit close examination of the extremely small lesion during endoscopic resection in the present case, a “strip biopsy” technique\textsuperscript{11,12} and two-channel endoscope were chosen. With this method, continuous visualization is possible during endoscopic resection. No residual lesion was found on follow-up endoscopy, and the absence of tumor was confirmed histologically. No major complications of the EMR procedure were noted, but longer follow-
up will be needed to confirm that EMR was curative.

The staining method described here is safe, relatively inexpensive, and suitable for investigating Barrett’s epithelium, including esophageal carcinogenesis. Systematic clinical trials of this double staining method coupled with EMR should be undertaken in patients suspected to have early-stage cancer or high-grade dysplasia in Barrett’s esophagus.

**REFERENCES**


