

Original Article

Delayed specimen collection may artifactually damage the mucosal surface in endoscopic mucosal resection specimens from Barrett's esophagus

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Abstract: Endoscopic mucosal resection (EMR) has become the standard of care for non-advanced Barrett's neoplasia; however, surface mucosal damage/artifact is a common observation in EMR specimens. Because surface maturation is an important morphologic consideration when analyzing dysplasia, this artifact can be problematic in selected cases. The exact cause of this mucosal surface damage has not been clearly delineated. We hypothesize that delayed specimen retrieval may be one cause of the mucosal surface damage observed in Barrett's EMR specimens. From the pathology archives of 2 institutions, 46 EMR specimens for dysplasia arising in Barrett's esophagus were retrieved. In 26 cases, the endoscopists immediately removed each portion of resected mucosa and placed it in fixative. In 20 other cases, the resected mucosal fragments were allowed to fall into the stomach and were retrieved at the end of the procedure. All the cases were reviewed by at least 2 gastrointestinal pathologists. Surface detachment (assessing mechanical injury related to the resection devices) and surface chemical injury (assessing acid/enzymatic injury from immersion in gastric contents) were scored in a semi-quantitative manner and analyzed with the Fisher's exact test. The specimens that were collected immediately upon resection demonstrated significantly less surface chemical injury (acid/enzymatic damage) compared to those with delayed collection ($p=0.001$). The degree of mechanical detachment was similar between the 2 groups ($p=0.2$). In conclusion, delayed specimen collection may cause significant mucosal surface damage and artifact in Barrett's EMR specimens. It is important to raise awareness of this avoidable pitfall among both endoscopists and pathologists.

Keywords: Artifact, Barrett's esophagus, endoscopic mucosal resection

Introduction

Barrett's esophagus (BE) is the only identified precursor lesion and the most important risk factor for the development of esophageal adenocarcinoma. The BE-dysplasia-adenocarcinoma sequence has been well established [1, 2]. For Barrett's associated high-grade dysplasia, the American Gastroenterological Association recommends endoscopic eradication therapy rather than surveillance [2]. Among all the endoscopic therapeutic methods, only endoscopic mucosal resection (EMR)/endoscopic submucosal dissection allows for both removal of tissue and accurate histopathologic diagnosis [2, 3]. EMR is strongly recommended and widely used in the United States for Barrett's dysplasia with a visible mucosal irregularity/nodularity to determine the pathologic T stage

of the neoplasm [2]. Although compared with endoscopic biopsies obtained with forceps, EMR specimens are large and well-oriented, and therefore allow easier assessment of dysplasia, there are still pitfalls when analyzing EMR specimens.

As EMR has become the standard of care for non-advanced nodular Barrett's neoplasia, there has been increasing recognition among surgical pathologists of the surface damage/artifact that commonly occurs as a result of the procedure. The exact causes of such observed mucosal surface damage have not been well studied. Because surface maturation is one of the most important morphologic features when evaluating dysplasia, developing clinical practice that allows minimal surface damage to EMR specimens is of great importance.

Artifacts in Barrett's EMR specimens

Table 1. Summary of the scores reflecting the degrees of surface mechanical detachment and chemical injury

Score	Surface Detachment		Surface Injury	
	Immediate collection	Delayed collection	Immediate collection	Delayed collection
0	9	5	21	6
1	12	14	4	7
2	5	1	1	7
Total	26	20	26	20
p-value	0.20		0.0015	

Some experts believe it is the plastic suction cap applied to the surface of the mucosa that causes surface damage, but this theory has never been proven by a systematic study. We hypothesize that delayed specimen retrieval may be one of the causes of mucosal surface damage, because endoscopists may allow the resected mucosa to fall into the stomach and retrieve the specimens at the end of the procedure. Theoretically, the acid and enzymes in the gastric juice may damage the devitalized specimen and consequently result in histological artifact. Our goal was to assess the relationship between EMR technique and extent of mucosal damage noted in pathology specimens.

Materials and methods

The pathology/clinical databases of the University of Chicago and Duke University Medical Center were searched, and specimens from EMR procedures for Barrett's esophagus were identified. The study was approved by the Institutional Review Boards at both institutions. Technical details of each endoscopic procedure were obtained from the endoscopic reports and confirmed by endoscopists. The slides from every case were reviewed by at least 2 gastrointestinal pathologists in a masked manner, and the following two parameters were scored in a semi-quantitative manner:

Surface detachment score: Areas of surface epithelial detachment devoid of inflammatory response were regarded as artifact caused by mechanical damage related to the use of the resection devices. These areas were scored as: 0 (none); 1 (focal surface epithelial detachment, less than one 40X field); 2 (large area of

surface detachment, greater than one 40X field).

Surface injury score: Areas of surface mucosal injury with necrotic epithelial debris and coagulative-like change, but devoid of acute inflammatory response, were regarded as artifact caused by chemical (acid/enzymatic) injury from immersion in gastric juice. These areas were scored as: 0 (none); 1 (injury limited to the surface epithelium); 2 (injury extending to involve the lamina propria).

Importantly, areas of ulceration or erosion where an inflammatory exudate was present were excluded and not scored for either parameter.

Data analysis & statistical considerations

Fisher's exact test was used to compare the surface detachment/injury scores between different groups.

Results

Forty-six consecutive cases were collected. In 20 cases, the resected mucosa was allowed to fall into the stomach and was not retrieved until the end of the procedure (delayed collection group). Because of the retrospective nature of this study, the exact time durations for which the specimens were left in the stomach were not available. In 26 other cases, the endoscopists immediately removed each portion of resected mucosa and placed it in fixative (immediate collection group).

Standard white light and narrow band imaging endoscopy were performed prior to EMR. No topical sprays (such as acetic acid) were used in any of the cases. Snare only technique was used in 2 patients, both in the immediate collection group. The remaining 44 patients all underwent cap-assisted mucosal resection.

Table 1 scores reflecting the degrees of surface chemical injury and mechanical detachment are summarized. Representative photomicrographs are shown in **Figure 1**. The specimens that were collected immediately demonstrated significantly less surface chemical (acid/enzymatic) injury compared to those with delayed collection ($p=0.0015$). In the immediate collection group, only 19.2% (5/26) of the cases ex-

Artifacts in Barrett's EMR specimens

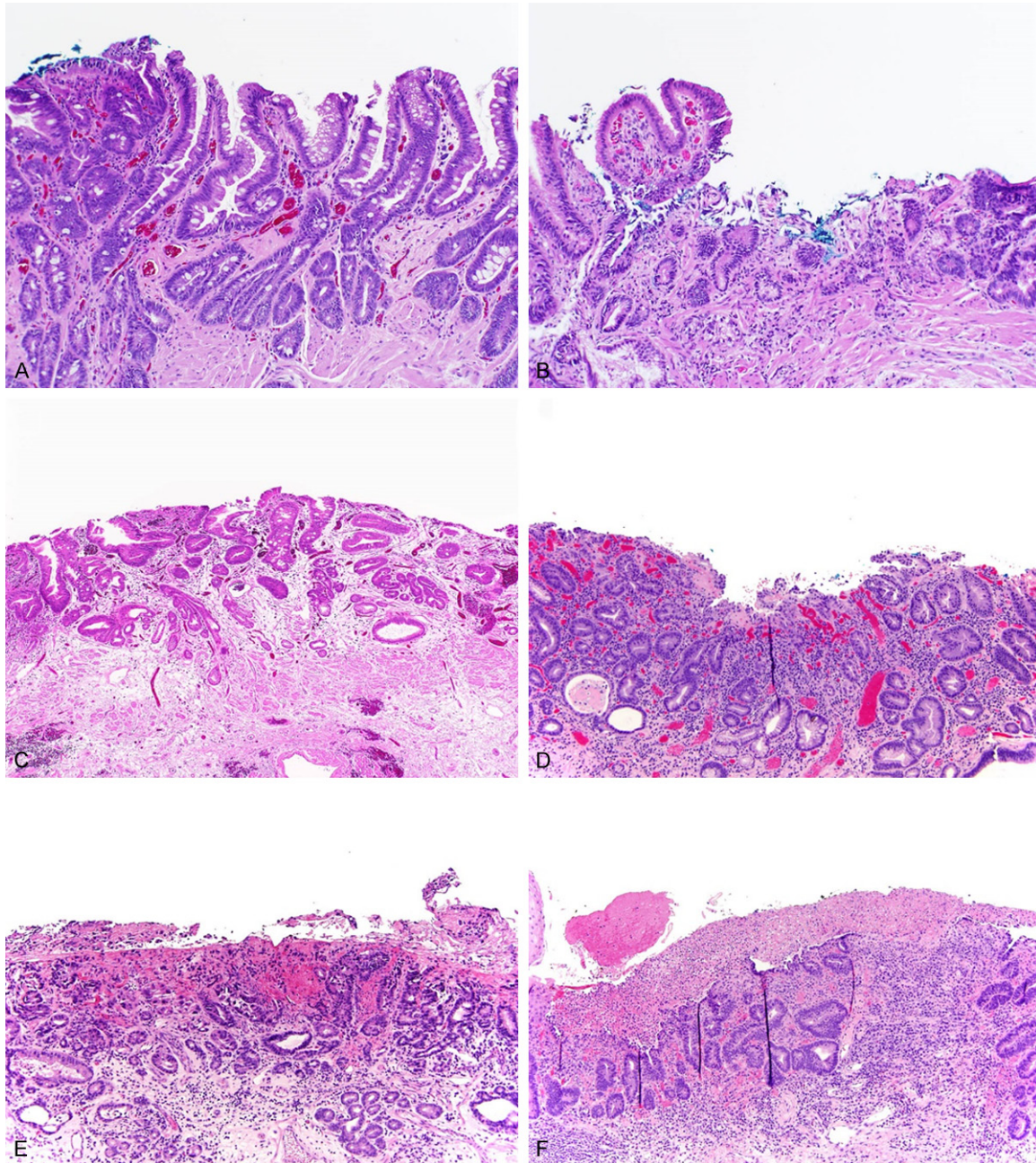


Figure 1. Examples of surface mucosal damage and artifact. A. Intact mucosal surface devoid of damage or artifact (Score 0). B, C. Surface detachment artifact, which is likely related to mechanical damage resulted from scope trauma and/or handling. This is defined as surface epithelial detachment devoid of inflammatory response. B. Score 1, focal surface epithelium detachment; C. Score 2, large areas of surface detachment. D, E. Surface injury artifact, which is likely caused by chemical (acid/enzymatic) injury from immersion in gastric juice. This is defined as mucosal injury with necrotic epithelial debris and coagulative type necrosis, but devoid of acute inflammatory response. D. Score 1, injury limited to the surface epithelium; E. Score 2, injury extending to involve lamina propria. F. Example of mucosal ulceration. Note that acute inflammation and inflammatory exudate seen in this example are not present in the cases with mechanical or chemical artifact.

hibited some degree of surface acid/enzymatic injury, and only 1 case exhibited grade 2 mucosal chemical injury. In contrast, surface acid/enzymatic injury was present in 70.0% (14/20)

of the specimens that were allowed to sit in the stomach for a period of time, and one half of these cases demonstrated grade 2 chemical mucosal injury.

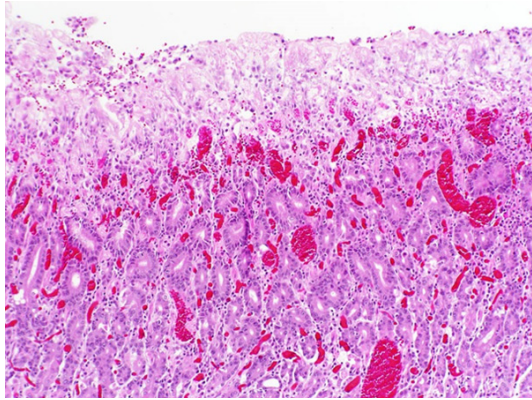


Figure 2. A partial gastrectomy specimen was stored at 4 °C before being fixed in formalin. Surface mucosal chemical injury was observed, secondary to the loss of defense mechanisms against autodigestion after being cut off from the blood flow.

Some degree of surface epithelial detachment (mechanical damage) was present in 69.6% of the cases, regardless of the retrieval method, and the degree of mechanical detachment was similar between the 2 groups ($p=0.20$, see **Table 1**). The specimens that were resected with snare technique exhibited minimal surface detachment; however, since snare technique was only used in 2 cases, statistical analysis was not possible due to the limited sample size.

Discussion

The EMR procedure removes Barrett's neoplasia with a diathermic snare down to the sub mucosa, providing large tissue specimens that can be easily oriented to accurately assess the depth of neoplastic involvement and the adequacy of the resection [4]. Thus, EMR has value as both a diagnostic/staging procedure and a therapeutic procedure for removing Barrett's epithelium with and without neoplasia. EMR is typically used for removal of lesions smaller than 2 cm [4]. For larger lesions, piecemeal removal is usually performed. Although small pieces of specimen are readily retrieved through the suction/biopsy channel of the endoscope, larger pieces (>1 cm) generally need to be retrieved with a net. In this latter scenario, it is relatively time-consuming to retrieve each portion of the specimen as it is resected, and a considerable amount of time can be saved by allowing multiple resected fragments to fall into the stomach followed by retrieval at the conclusion of the procedure.

Our data indicated that delayed specimen retrieval may cause significant mucosal surface damage and artifact. Theoretically, tissue damage and artifact may occur even if the specimen is immersed in gastric juice for a short period of time. Under physiologic conditions, gastric mucosa maintains structural integrity and resists autodigestion by a number of defense mechanisms including pre-epithelial factors (mucus-bicarbonate-phospholipid "barrier"), an epithelial "barrier" (surface epithelial cells connected by tight junctions and generating bicarbonate, mucus, phospholipids, trefoil peptides, prostaglandins, and heat shock proteins), continuous cell renewal accomplished by proliferation of progenitor cells, and continuous blood flow through mucosal microvessels [5]. The intestinal type of Barrett's mucosa lacks many of the defense mechanisms of normal gastric mucosa. After being cut off from the blood flow, resected Barrett's mucosa is more susceptible to acid and pepsin digestion. As a matter of fact, even normal gastric mucosa is susceptible to chemical injury after being resected. This is exemplified by the occasional observation of surface chemical injury in stomach specimen being stored at 4°C before formalin fixation (**Figure 2**). Even in patients treated with proton pump inhibitors, pepsin, bile, and pancreatic secretions may still be sufficient to injure Barrett's mucosa immersed in gastric contents.

Some experts believe it is the plastic suction cap device applied to the surface of the mucosa during the endoscopic resection that causes surface damage. Currently, the most commonly used techniques can be subdivided as injection-, cap-, and ligation-assisted EMR, with suction being used in the latter two methods [4]. Whether cap-assisted resection with suction causes tissue damage and artifact has not been studied in a systematic manner. Farrell JJ et al. compared cap-assisted EMR with standard snare EMR in a porcine model and reported that use of the cap was associated with less diathermic injury to the specimens, especially at the peripheral margins where the histological analysis is crucial [6]. However, the authors did not compare surface damage and artifact between the two groups. Our study demonstrates that surface detachment, which is likely a result of mechanical damage associated with the plastic cap, is common (63.4% in the imme-

diate collection group, and 75.0% in the delayed collection group), and does not differ depending on the retrieval methods. Given the popularity of cap-assisted resection, it would be difficult to modify this possible artifact-inducing factor until an equivalent alternative endoscopic technique becomes clinically available. In contrast, the superficial mucosal injury that occurs as a result of immersing resection fragments in gastric juice can easily be avoided by immediately retrieving each portion of the specimen as it is resected (even though this will increase the overall length of the procedure). Because this study was retrospective, we do not know the duration for which the resection fragments were immersed in gastric juices in the delayed collection group. Our sense is that the mucosal injury can develop in a relatively short period of time and that it is best to completely avoid delayed retrieval. Another limitation of this study is that we did not quantify to what degree delayed specimen retrieval causes diagnostic difficulty and disagreement. For reasons discussed above, immediate specimen retrieval should be performed even if delayed retrieval only gives rise to diagnostic challenge in a small proportion of cases.

In summary, delayed specimen collection may cause significant mucosal surface damage and artifact in Barrett's EMR specimens. It is important to raise awareness of this avoidable pitfall among both endoscopists and pathologists.

Disclosure of conflict of interest

None.

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