Cardiac mucosa in the remnant esophagus after esophagectomy is an acquired epithelium with Barrett’s-like features

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Background. The cervical esophagus is normally lined by squamous epithelium and is usually not exposed to gastroesophageal reflux. The aims of this study were, first, to investigate whether cardiac mucosa can be acquired in the remnant cervical esophagus after esophagectomy and cervical esophagogastrostomy and, second, to characterize this mucosa if present.

Methods. The medical records of 100 patients who had undergone esophagectomy with gastric pull-up reconstruction were studied retrospectively to identify those who had biopsies from the cervical esophagus proximal to the gastroesophageal anastomosis during postoperative follow-up. The histopathology and immunohistochemical stains were reviewed to assess similarity to Barrett’s mucosa (cytokeratins [CK] 7 and 20 and DAS-1), cellular proliferation (topoisomerase 2α), and the potential for dysplasia (cyclooxygenase 2 [COX-2] and ornithine decarboxylase [ODC]).

Results. Supra-anastomotic biopsies were performed in 20 patients. Cardiac mucosa was present in 10 of 20 (50%) patients in whom biopsies were performed. Four patients had areas of intestinal metaplasia, and dysplasia, and adenocarcinoma developed in 1 patient. The CK7/20 and DAS-1 staining of the columnar mucosa showed a pattern similar to Barrett’s mucosa. Topoisomerase 2α protein expression was present in 50% of patients with cardiac mucosa. DAS-1 protein was expressed in cervical columnar mucosa but not in normal squamous esophageal mucosa. The cardiac mucosa stained weakly for COX-2 and ODC.

Conclusions. Cardiac mucosa can be acquired. Its expression profile is similar to cardiac mucosa and intestinal metaplasia found in Barrett’s esophagus, and different from normal esophageal or gastric mucosa. The development of cardiac mucosa is likely to be related to reflux of acid into the remnant cervical esophagus as the first step in the development of Barrett’s esophagus. These findings are applicable to the development of similar changes at the gastroesophageal junction. (Surgery 2004;136:633-40.)

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Cardiac mucosa is a simple, mucinous columnar mucosa with foveolar hyperplasia and no parietal cells. It is found in the region of the gastroesophageal junction in most adults in Western society. When present, it is almost invariably accompanied by an infiltrate of chronic or acute inflammatory cells and may thus be termed “carditis.” Cardiac mucosa is distinguished from the intestinal metaplasia (IM) that characterizes Barrett’s esophagus only by the absence of goblet cells.

In the past, it was believed that up to 2 cm of cardiac mucosa was normally present in the most proximal section of the stomach, where it separates the parietal cell-containing gastric oxyntic mucosa from the esophageal squamous mucosa. This prevailing view was challenged by a study suggesting that cardiac mucosa, rather than being a normally occurring mucosa, might be an acquired,
metaplastic epithelium that develops in response to exposure of esophageal squamous epithelium to gastric acid. According to this hypothesis, the histology of the normal gastroesophageal junction consists of squamous mucosa abutting the parietal cell-containing gastric oxyntic mucosa. Subsequent studies have confirmed that this histologic pattern, with squamous epithelium directly abutting oxyntic, does occur. Further observations suggest that the development of cardiac mucosa is induced by exposing squamous epithelium to refluxed gastric acid. The stimulus for the development of cardiac mucosa may be refluxed gastric acid. This introduced the possibility that the formation of cardiac mucosa may be the first step in the development of Barrett’s esophagus. Others reject this possibility, leading to controversy regarding the nature and etiology of cardiac mucosa. Unfortunately, limitations as to the accurate location of endoscopic biopsies and the rapid autolysis of the mucosa of the gastroesophageal junction in autopsy specimens have made it difficult to resolve this controversy.

In this study, we used the cervical esophagus after esophagectomy reconstructed with a gastroesophagostomy as a model for de novo gastroesophageal reflux. In this situation, it can be confirmed histologically that the squamous-lined cervical esophagus is anastomosed to the gastric fundus lined with oxyntic mucosa because any previously present cardiac mucosa was removed with the surgical specimen. Further, the cervical esophagus is not normally exposed to gastric juice. Even in patients diagnosed with gastroesophageal reflux disease (GERD), acid exposure in the cervical esophagus is relatively infrequent—less than 1% of a 24-hour period. Consequently, even in patients with severe GERD, the cervical esophagus is normally lined by squamous epithelium. In contrast, after esophagectomy and gastric pull-up, free reflux into the cervical esophagus occurs. Esophagectomy and cervical esophagogastrostomy is thus a unique in vivo human model for de novo reflux disease.

This study was undertaken to test the hypothesis that cardiac mucosa is an acquired, metaplastic epithelium that arises from squamous mucosa in response to exposure to gastric acid. If this hypothesis is correct, reflux into the cervical esophagus after esophagectomy should result in the development of cardiac mucosa in the remnant esophagus, as reported in other studies. We also sought to characterize with immunohistochemistry the acquired mucosa and to compare it with cardiac mucosa at the gastroesophageal junction and Barrett’s mucosa in the distal esophagus.

MATERIAL AND METHODS

After obtaining approval for this study from the Institutional Review Board of the University of Southern California Keck School of Medicine, the medical records of 100 patients with esophageal adenocarcinoma or squamous cell carcinoma who had undergone esophagectomy with gastric tube reconstruction were retrospectively reviewed. Patients who had received chemotherapy or radiation therapy were excluded because of the reported association between these treatments and the development of Barrett’s esophagus and esophageal cancer. Of the 100 patients, 20 had an endoscopic evaluation, which included a biopsy of the remnant cervical esophagus taken from above the gastroesophageal anastomosis 9 months or more after esophagectomy. The endoscopies were performed to investigate symptoms, including regurgitation, dysphagia, chest pain, and either loss or failure to gain weight; thus, these patients were somewhat selected and may not be representative of the entire group of 100 patients reviewed. The supra-anastomotic biopsies were performed to conduct studies such as the present one. All biopsies were performed by members of the surgery faculty. The hematoxylin and eosin (H&E) stained slides of all postoperative endoscopic biopsies from these 20 patients were reviewed, and patients with cardiac mucosa in the cervical esophagus were identified. Formalin-fixed, paraffin-embedded blocks of the esophageal biopsies and the gastric biopsies, if obtained, from the patients with cardiac mucosa in the cervical esophagus were retrieved from the pathology archives. In these selected patients, the pathology report on the surgical margin before anastomosis was reviewed.

**Histopathology.** The H&E-stained slides of the supra-anastomotic cervical esophageal biopsies were examined using the criteria of Paull et al. as modified by Chandrasoma et al. The diagnosis of pure cardiac mucosa was made when the glands were composed of mucous cells only, with no parietal cells. Pure cardiac mucosa is thus similar to the junctional epithelium of Paull et al. Oxyntic mucosa was recognized by the presence of glands containing both mucous and parietal cells, equivalent to the fundic epithelium described by Paull et al. The diagnosis of IM required the presence of definitive goblet cells. When visible macroscopically, IM (or specialized epithelium) is referred to as Barrett’s esophagus. Information in the medical records was insufficient to be certain of the endoscopic appearance of the cervical esoph-
agus in some patients, and therefore, macroscopic data were not collected.

**Immunohistochemistry.** Immunohistochemical stains were performed to assess similarity to Barrett’s mucosa (cytokeratins [CK] 7 and 20 and DAS-1), cellular proliferation (topoisomerase 2α), and the potential for dysplasia (cyclo-oxygenase 2 [COX-2], and ornithine decarboxylase [ODC]). Archival formalin-fixed, paraffin-embedded blocks were cut into 6-μm sections, mounted onto polylysine-coated slides, dewaxed in xylene, and rehydrated through graded alcohol steps at room temperature. Pretreatment by immersion in 10 mmol/L citrate buffer pH 6.0 with microwave, pressure cooker heating was performed for all the antibodies used. A 0.05M Tris-HCl buffer solution (pH 7.6) was used to prepare solutions and for washes between steps. The sections were peroxidase-blocked using 3% hydrogen peroxide in 0.05 mol/L TRIS-hydrochloric acid buffer, incubated for 15 minutes with normal horse serum, and incubated with primary antibody (all overnight at room temperature). The primary antibodies used were: topoisomerase 2α Mab (diluted 1:100; Neomarkers, Clone JH2.7, Fremont, Calif), cytokeratin (CK) 7 and CK 20 (both 1:100; DAKO, Carpinteria, Calif), ornithine decarboxylase (ODC-29, 1:50; Sigma Chemical, St. Louis, Mo), DAS-1 (1:5, kindly provided by Dr Kiron M. Das, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School), and cyclooxygenase 2 Mab (COX-2 clone 33, 1:50; Transduction Laboratories, Lexington, Ky). Biotinylated horse antimouse secondary antibody (1:200 dilution for 40 minutes; Vector Labs, Burlingame Calif), peroxidase-conjugated-streptavidin complex reagent (1:100 dilution, 30 minutes, VectaStain Elite ABC Kit, Vector Labs), and 3,3′-diaminobenzidine (DAB, 10 mg in 10 ml tris buffer for 20 minutes) were used to visualize binding of the first antibody. Positive controls included sections of colon cancer (CK-20), breast cancer (CK-7), lymph node (topoisomerase 2α), and normal colon (DAS-1). Negative controls used the study sections without primary antibody. Immunoreactivity was graded as positive when there was moderate or strong staining of at least 5% of the mucosal cells of interest.

**RESULTS**

Cardiac mucosa was present in the cervical esophagus in 10 of the 20 (50%) patients who underwent biopsy. The indications for esophagectomy in these 10 patients were adenocarcinoma in 7, squamous cell carcinoma in 2, and stricture in 1. In 4 of the 7 patients with adenocarcinoma, IM was identified in the esophagectomy specimen. In all patients, the surgical margins prior to anastomosis showed no IM or cardiac mucosa. Figure 1 shows the endoscopic appearance of an area of IM in the cervical esophagus in 1 patient.

Seven of the 10 patients who showed cardiac mucosa on biopsy of the remnant cervical esophagus were males, and the median interval between esophagectomy and biopsy of cardiac mucosa was 36 months (range, 9 months–42 years). Cardiac mucosa was found on the first endoscopy after esophagectomy in 9 patients. These are the only biopsies that were available for these patients. One patient had 2 post-esophagectomy endoscopies with biopsy. At the first endoscopy, performed 15 months after esophagectomy, only squamous epithelium was present in the biopsy of the cervical esophagus. At the second endoscopy, performed 9 months later, cardiac mucosa was found in the cervical esophagus.

Four of the 10 patients with cardiac mucosa also had goblet cells characteristic of IM in the supra-anastomotic biopsies. One of the 4 patients, a
Table. Immunohistochemistry findings in different tissue types from the 10 patients with cardiac mucosa in the remnant cervical esophagus

<table>
<thead>
<tr>
<th>Immunohistochemistry stain</th>
<th>Purpose</th>
<th>Normal squamous mucosa</th>
<th>Normal gastric antral mucosa</th>
<th>Cardiac mucosa</th>
<th>Cardiac mucosa with IM</th>
<th>Cardiac mucosa with IM, dysplasia, adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td></td>
<td>10</td>
<td>5</td>
<td>10</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>CK 20</td>
<td>No superficial staining.</td>
<td>Superficial staining in 2/5.</td>
<td>No deep glandular staining.</td>
<td>Superficial staining in all.</td>
<td>No deep glandular staining.</td>
<td>Superficial staining in all. No deep glandular staining.</td>
</tr>
<tr>
<td>DAS-1</td>
<td>No staining.</td>
<td>Staining of oxynic cells.</td>
<td>Weak cytoplastic staining of columnar cells in 2/10.</td>
<td>Staining in all with more intense staining in goblet cells than in cardiac mucosa.</td>
<td>Staining of mucin within goblet cells.</td>
<td>Staining of mucin within goblet cells.</td>
</tr>
<tr>
<td>Topoisomerase 2α</td>
<td>Cellular proliferation</td>
<td>Staining in basal layer.</td>
<td>Staining of basal layer of crypts and superficial glands.</td>
<td>Staining in all with more intense staining in goblet cells than in cardiac mucosa.</td>
<td>Intense staining in dysplastic and tumor cells.</td>
<td>Staining.</td>
</tr>
</tbody>
</table>

CK, Cytokeratin; COX, cyclo-oxygenase; ODC, ornithine decarboxylase.

*Excludes staining of inflammatory cells.

A 57-year-old man who had undergone esophagectomy at age 15 for an esophageal stricture secondary to ingesting a coin at 9 months of age, had Barrett’s esophagus with dysplasia and an intramucosal adenocarcinoma in the remnant cervical esophagus 42 years after esophagectomy.

Immunohistochemistry. The immunohistochemistry results are shown in the Table. Representative images are shown in Figures 2, 3, 4, 5, and 6. CK7 staining of the cervical esophagus with cardiac mucosa, IM, and dysplasia was similar to Barrett’s esophagus in the distal esophagus and was seen consistently in both the surface epithelium and the deep glandular cells (Fig 2, A). The staining was typically more intense in areas of IM and dysplasia. In contrast to the columnar metaplasia pattern, CK7 staining of squamous mucosa showed focal staining in the deep glandular cells without staining in the superficial squamous cells. Also similar to the pattern seen in Barrett’s esophagus, CK20 staining of the cervical esophagus containing columnar mucosa occurred in the surface cells, but there was little or no staining of the deep glandular cells (Fig 2, B). In contrast, squamous mucosa did not stain. Again as found in Barrett’s esophagus, DAS-1 antibody in cervical cardiac mucosa stained intensely the mucin within goblet cells and faintly the cytoplasm of some of the columnar cells (Fig 3). Squamous epithelium did not stain. All the cervical cardiac mucosa, including sections with IM and dysplasia, showed positive topoisomerase 2α staining, which was typically stronger at the bases of crypts and in glands than in the surface epithelium (Fig 4). The staining was stronger in goblet cells and dysplastic cells than in cardiac columnar cells. Normal squamous epithelium showed topoisomerase 2α staining in the basal layer.
The staining pattern for COX-2 and ODC was similar. In the cervical esophagus, cytoplasmic COX-2 epithelial staining was faint and was present in only 2 of 10 patients with cardiac mucosa (Fig 5). It was not present in those with IM but was present in the tumor cells in the patient with cancer. Intense ODC cytoplasmic epithelial staining was present in the cervical esophageal mucosa with dysplastic and tumor cells (Fig 6). Weak ODC cytoplasmic staining was present in 6 of 10 patients with cervical cardiac mucosa. It also was present in goblet cells in 3 of the patients with IM. Squamous mucosa did not stain for both antibodies, except for COX-2 stained inflammatory cells in the lamina propria.

**DISCUSSION**

Our study shows that cardiac mucosa can be an acquired, metaplastic epithelium. In the remnant cervical esophagus of patients who had esophagectomy with esophagogastrectomy, an operation that provides a unique human model for de novo reflux disease, we found cardiac mucosa in the supra-anastomotic biopsy in half the patients who had biopsy at this site. This indicates that the columnar mucosa in these selected patients was
acquired after the operation because at the time of surgery, oxyntic mucosa was anastomosed to squamous mucosa. The stimulus for this metaplastic process is almost certainly acid reflux into the remnant esophagus. This is supported by a study from Sweden by Oberg et al in which pH probes measuring 24-hour acid exposure were placed in the cervical esophagus 1 cm above the esophagogastric anastomosis in patients who had esophagectomy. All patients with columnar mucosa in the remnant esophagus had abnormal acid exposure, and there was a direct correlation between the length of the metaplastic segment and the percentage of time the cervical esophagus was exposed to a pH less than 4.0. Interestingly, there was no association between the presence of cervical columnar metaplasia and exposure to bilirubin, a marker for non-acid reflux, although the few patients with IM in the cardiac mucosa had abnormal esophageal exposure of both acid and bilirubin. These results are similar to those found in the distal esophagus, as indicated by a study that found that similar proportions of patients with cardiac mucosa and IM had abnormal esophageal acid exposure (79% and 83%, respectively), but abnormal esophageal bilirubin exposure was more frequent in the patients with IM. Based on these results, the hypothesis may be advanced that acid reflux is sufficient to stimulate the development of cardiac mucosa, but non-acid reflux may be particularly important for the development of IM.

We found that cardiac mucosa in the remnant esophagus shares some definitive characteristics with cardiac mucosa and Barrett’s esophagus in the distal esophagus. Most importantly, we found a CK 7/20 expression pattern similar to that of Barrett’s esophagus in a majority of the patients with cardiac mucosa with or without IM and dysplasia. In contrast, a “non–Barrett’s-like” CK pattern was present in specimens of normal squamous and gastric mucosa. The similarity between supra-anastomotic cardiac mucosa, distal esophageal cardiac mucosa, and Barrett’s esophagus supports the likelihood that IM may result from the development of goblet cells within the cardiac mucosa. Further, the “Barrett’s” CK staining pattern also has been shown to be associated with a reflux etiology. This study further supports the likelihood of a reflux etiology for cardiac mucosa in the remnant esophagus after esophagectomy.

The DAS-1 MAb reacts against colonic epithelial cells, but not with normal small-bowel enterocytes or esophageal mucosa. The antibody does react intensely with an unknown epitope in Barrett’s esophagus, particularly the incomplete (II and III) type of IM. In the present study, intense DAS-1 staining of the mucin in goblet cells was observed in cervical esophagus with intestinalized cardiac mucosa. Further, the pattern of DAS-1 staining in both the IM and cardiac columnar cells was similar to that found in the lower esophagus.

Cellular proliferation was assessed with topoisomerase 2α immunohistochemistry. As expected, the proliferative zones in normal gastric and esophageal mucosa, dysplastic Barrett’s cells, and cancer cells were strongly positive for topoisomerase 2α. There was also evidence of increased proliferation in some of the cardiac mucosa, suggesting the possibility of disease progression in some patients.
with non-IM columnar metaplasia. The protein expressions of ODC and COX-2 were also examined. Both of these genes have putative roles in tumorigenesis. ODC is the initial and rate-limiting enzyme in the biosynthetic pathway of polyamines, which have essential roles in cell growth and differentiation. Increased ODC protein and mRNA expression have been reported in Barrett’s esophagus and adenocarcinoma. Similarly, the prostaglandin synthesis enzyme COX-2, which has been implicated as a fundamental factor in many tumorigenic processes, is upregulated in some Barrett’s tissues. The low ODC and COX-2 expressions found in cardiac mucosa in this study support the clinical observation that cardiac mucosa has very little malignant potential.

Cardiac mucosa is frequently present at the gastroesophageal junction in adults in Western society, raising the possibility that we have merely taken biopsies of long-standing cardiac mucosa from the distal, gastric side of the anastomosis. This possibility is extremely unlikely because we included only patients in whom it was noted that the biopsies were from above the anastomosis, and because at least 2 cm of proximal stomach, and thus the gastroesophageal junction and all cardiac mucosa, was resected at the time of esophagectomy. The possibility that columnar mucosa was present in the cervical esophagus before esophagectomy is also excluded because only normal squamous epithelium was seen at the proximal resection margin at the time of operation. Furthermore, the supra-anastomotic biopsies were performed for the specific purpose of conducting studies such as the present one. In this respect, although the methods of data collection and specimen retrieval make this a retrospective study, the biopsies were collected prospectively.

In summary, cardiac mucosa can be an acquired, metaplastic epithelium. It is likely that it develops commonly after esophagectomy with gastric reconstruction and its presence very likely signifies at least some reflux into the esophagus. This observation supports the hypothesis that cardiac mucosa at the gastroesophageal junction in unoperated individuals, despite the prevalence of this finding, is also an acquired epithelium. CK 7/20 characterization shows that the cardiac mucosa in the remnant esophagus is similar to Barrett’s in the distal esophagus. This supports the possibility that IM could arise from cardiac mucosa. Dysplastic Barrett’s and adenocarcinoma developed in 1 patient in our study 42 years after esophagectomy. This observation does not indicate the need for routine post-esophagectomy surveillance of the remnant esophagus, except perhaps in long-term survivors.

REFERENCES


