Gland ducts and multilayered epithelium in mucosal biopsies from gastroesophageal-junction region are useful in characterizing esophageal location

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SUMMARY. There is controversy as to whether oxynto-cardiac mucosa (OCM), cardiac mucosa (CM) and intestinal metaplasia (IM) found in the gastroesophageal-junction region line the anatomic stomach, esophagus or both. A total of 785 retroflex biopsies taken at the endoscopic gastroesophageal junction in 244 patients were evaluated for the presence of gland ducts and multilayered epithelium which are two recognized markers of esophageal mucosa. Oxyntic mucosa was found in 287 biopsies, OCM in 283, CM in 158, IM in 30 and squamous epithelium in 53 (some biopsies had more than one epithelial type). Esophageal gland ducts and multilayered epithelium were absent in all biopsies with oxyntic mucosa. Sixty-four (13.6%) of 471 biopsies with OCM, CM and IM contained esophageal gland ducts and 68 of 471 (14.4%) contained multilayered epithelium. Ninety-eight of 471 (20.8%) biopsies contained either gland ducts or multilayered epithelium. This study shows that 20.8% of biopsies at the gastroesophageal junction with OCM, CM and IM can be definitively characterized as lining the anatomic esophagus by the finding of gland ducts and multilayered epithelium. The absence of these markers in oxyntic mucosa confirms this epithelium as gastric. The presence of gland ducts and multilayered epithelium can be used by pathologists to objectively ascribe an esophageal or gastric location to a biopsy from the gastroesophageal junction.

KEY WORDS: Barrett’s esophagus, cardiac mucosa, gastroesophageal junction, gastroesophageal reflux, histology, intestinal metaplasia.

INTRODUCTION

Paull et al. in their classic histologic study,18 reported oxynto-cardiac mucosa (OCM), cardiac mucosa (CM) and intestinal metaplasia (IM) as being the three histologic types of columnar lined esophagus (they used the synonymous terms fundic, junctional and specialized, respectively, for these epithelia). We have confirmed that these three glandular epithelia, esophageal squamous epithelium and gastric oxyntic mucosa are the only epithelial types occurring in the foregut between the proximal esophagus and gastric antrum.1,5 Other cells types, such as Paneth cells, serous cells and pancreatic cells can occur within these epithelial types. We have recently suggested that OCM, CM and IM are exclusively esophageal, being derived from transformation of the reflux-damaged squamous epithelium of the esophagus.1–4 Other authorities opin that the more traditional viewpoint that OCM and CM occur as normal or native epithelia in the stomach is correct.10,11,16

Defining the stomach and esophagus is crucial in diagnosing diseases of the gastroesophageal region. While the presence of oxyntic mucosa indicates that the biopsy is from the stomach and stratified squamous epithelium defines a biopsy as esophageal, there is no histologic method to characterize a biopsy containing OCM, CM and IM as being esophageal or gastric. At present, the pathologist is dependent on the gastroenterologist’s definition of the gastroesophageal junction and the biopsy site relative to this point to define it as being gastric and esophageal. Thus, if IM is found in a biopsy distal to the endoscopically defined gastroesophageal junction, it represents ‘intestinal metaplasia of the gastric cardia’, while if the endoscopist...
declares this biopsy to be proximal to the junction, it will represent Barrett’s esophagus.

In the normal patient, the proximal limit of the gastric rugal folds, which is regarded as the most reliable endoscopic marker of the gastroesophageal junction, closely approximates the esophageal squamous epithelium. With increasing reflux, the squamo-columnar junction moves proximally as a result of glandular transformation of esophageal squamous epithelium. When sufficiently separated from the proximal limit of the rugal folds, this abnormal esophageal glandular mucosa can be recognized at endoscopy as a flat, inflamed mucosa that is different to the gastric oxyntic mucosa. When biopsies are taken from this abnormal flat mucosa between the proximal limit of the rugal folds and squamous epithelium, Paull et al.’s three epithelial types are found. When IM is found in a biopsy taken from this endoscopically visualized abnormal columnar epithelial lining, Barrett’s esophagus is diagnosed.

However, when biopsies are taken from the gastroesophageal junctional region in patients who have no visible endoscopic abnormality, there is a marked variation in the frequency with which OCM, CM and IM are found. Controversy exists as to whether OCM, CM and IM in the region of the gastroesophageal junction in patients with no endoscopic abnormality represent the lining of the stomach or glandular transformation of the esophagus.

The ducts of esophageal glands and multilayered epithelium are two recognized histologic markers of esophageal mucosa. This study aims to document the prevalence and distribution of esophageal gland ducts and multilayered epithelium in the different glandular epithelial types in mucosal biopsies taken in the region of the gastroesophageal junction.

**MATERIALS AND METHODS**

Two hundred and forty-four consecutive patients, irrespective of the nature of symptoms, who had a retroflex biopsy taken at the proximal limit of the rugal folds were selected. Endoscopy was performed by faculty of the University of Southern California Foregut Surgery Department, using Olympus video endoscopes (Olympus America, Inc, Melville, New York), and biopsies were obtained with standard biopsy forceps (Microvasive radial jaw 31263–20 or 1597–20). Each retroflex biopsy specimen consisted of 2–4 biopsies with a total of 785 biopsies taken in the 244 patients (mean, 3.2 biopsies per patient). The retroflex biopsies were obtained according to a standard biopsy protocol that was used irrespective of the presence or absence of an endoscopic abnormality. When the patient had no endoscopic abnormality, the retroflex biopsy represented sampling of the glandular mucosa at the proximal limit of the rugal folds and the squamo-columnar junction. When the patient had a visibly abnormal flat glandular mucosa between the proximal limit of the rugal folds and the squamous epithelium, the retroflex biopsy represented sampling of the gastroesophageal junction defined as the proximal limit of the gastric rugal folds. In these patients additional biopsies were taken at 1–2 cm intervals from the more proximal columnar lined esophagus. Only the retroflex sample was used for this study because it would have the highest yield of mucosal types at the endoscopically defined gastroesophageal junction. In all patients, the area under study by these retroflex biopsies is the mucosa within 0.5 cm of either side of the proximal limit of the gastric rugal folds.

The glandular epithelia found in the retroflex biopsies were classified into oxyntic mucosa, OCM, CM and IM using previously published criteria. Cardiac mucosa was defined by the presence of only mucous cells in the epithelium. Oxyto-cardiac mucosa contained a mixture of mucous cells and parietal cells in the glands. Intestinal metaplastic mucosa was defined by the presence of well defined goblet cells in the glands, foveolar region or surface epithelium in hematoxylin and eosin stained sections. Some biopsy samples contained more than one epithelial type; these were recorded separately.

Esophageal gland ducts are readily recognizable in the lamina propria as rounded or elongated structures lined by a stratified basaloïd squamous epithelium with or without a surface columnar cell layer that surrounds a lumen (Figs 1, 2). Intercellular bridges are frequently present in the squamous epithelium. The squamous lined ducts open at the surface of the mucosa, retaining their characteristics.
up to the surface (Fig. 3). The columnar epithelial surface layer is usually simple, but can rarely be ciliated (Figs 4, 5). The presence of esophageal gland ducts and which mucosal type they occurred in was recorded.

Multilayered epithelium occurs predominantly in the surface epithelium, although rarely it can be seen in the superficial foveolar region. It consists of multiple layers of cells with basaloid squamous cells in the basal region and a columnar epithelial cell layer overlying the basal cells (Figs 6, 7).
presence of multilayered epithelium and which mucosal type it occurred in was recorded.

RESULTS

The distribution of epithelial types in the 785 biopsy samples from the 244 patients is shown in Table 1. A total of 732 biopsies consisted of glandular mucosa; 53 biopsies were composed entirely of squamous epithelium. Some biopsies contained more than one type of glandular mucosa and these were counted separately. Oxyntic mucosa was the most frequent epithelial type found (287 biopsies), followed by OCM (283), CM (158) and IM (30).

Esophageal gland ducts were present in 64/732 biopsies with glandular mucosa (8.7% of total and 13.6% of glandular mucosa excluding oxyntic mucosa), being present in 25/283 (8.8%) biopsies with OCM, 34/158 (21.5%) biopsies with CM (Fig. 6), and 9/30 (30.0%) biopsies with IM. Multilayered epithelium was not present in oxyntic mucosa (Table 1).

Some biopsies contained both gland ducts and multilayered epithelium. The total number of separate biopsies that had either gland ducts or multilayered epithelium was 98/732 biopsies with glandular mucosa (13.4% of the total and 20.8% of glandular mucosa excluding oxyntic mucosa).

DISCUSSION

The submucosal glands of the esophagus drain to the squamous epithelial surface via ducts that are lined either by squamous cells or a mixture of squamous and columnar cells. These submucosal glands and their ducts remain even after the surface squamous epithelium becomes transformed into glandular epithelium, and represent a marker for the anatomic esophagus because they are not found in the stomach.8 We have made anecdotal reference previously to the presence of these ducts in CM and point to their presence in CM as evidence that CM lines the esophagus rather than the stomach.6 It should be noted that submucosal gland numbers vary markedly in different individuals and gland ducts are randomly found in the mucosa of the esophagus. While their presence marks the mucosa as esophageal, no conclusion can be drawn by their absence.

Glickman et al. reported the presence in the esophageal transformational zone of a transitional multilayered epithelium with mixed squamous and glandular elements.12 This multilayered epithelium has been suggested to represent an intermediate stage in the transformation of squamous to columnar epithelium in the esophagus.12 There appears to be little argument that multilayered epithelium is also a marker of the reflux-damaged esophageal mucosa because it is not present in gastric oxyntic mucosa.
The concept of normal histology of the gastroesophageal junctional region has undergone significant revision in the recent past. The traditional view that cardiac mucosa lines the proximal stomach and 2–3 cm of the distal esophagus has been proved incorrect by autopsy studies. Chandrasoma et al. reported the absence of cardiac mucosa in 56% of autopsies. Kilgore et al., in an autopsy study of children, reported that the mean length of cardiac mucosa between the squamous epithelium and gastric oxyntic mucosa was only 0.18 cm (range: 0.1–0.4 cm). Derdoy et al., in an autopsy study that includes premature babies, reported a mean length of CM of 0.1 cm (range 0.01–0.3 cm) between squamous and oxyntic mucosa. This evidence indicates that CM is normally either absent or present in a very small length at the gastroesophageal junction.

In several recent papers, we have hypothesized that oxyntic mucosa lines the entire proximal stomach, squamous epithelium lines the normal esophagus, and that the presence of OCM, CM and IM indicate reflux-damaged esophagus with glandular transformation of the squamous epithelium. It is accepted that endoscopically visualized abnormal columnar lined esophagus with intestinal metaplasia is Barrett’s esophagus and therefore caused by reflux. We have postulated that the presence of OCM, CM and IM represents reflux-induced changes in the esophagus even in patients with no endoscopic abnormality. The finding of OCM, CM and IM in endoscopically normal patients is simply a reflection of the superiority of microscopic examination over gross and endoscopic examination in identifying mucosal changes. According to our hypothesis, OCM, CM and IM are never normal ‘native’ mucosae of the stomach. In patients without symptoms of reflux, the finding of OCM, CM and IM in biopsies indicates subclinical histologic evidence of reflux-induced damage to squamous esophageal lining. In patients without reflux damage, OCM, CM and IM will be absent in the junctional region with mucosal biopsies showing only oxyntic (gastric) and squamous (esophageal) epithelium. The opposing viewpoint maintains the traditional viewpoint that CM is a normal mucosal lining of the proximal stomach.

When biopsies are taken from patients who have no visible endoscopic abnormality, there is a marked variation in the types of epithelia that are present. Jain et al. found CM to be absent in 65% of endoscopically normal patients who underwent extensive biopsy sampling of this region. Glickman et al. reported the absence of CM in 19% of a pediatric population, many with symptoms of reflux but reported to be endoscopically normal. Oberg et al. found CM and OCM to be absent in 26% of patients in a study of 344 patients. In Oberg et al.’s study, the presence of CM and OCM was associated with a greater amount of acid reflux than in patients who did not have CM and OCM, providing strong evidence that CM and OCM are reflux-induced abnormal epithelia when found by biopsy in endoscopically normal individuals. Der et al. reported that CM, when found, was always inflamed and the amount of inflammation was significantly related to acid exposure. This suggests that CM is damaged by reflux.

In the present study, esophageal gland ducts were present in 13.6% of biopsies with OCM, CM and IM. At least in these biopsies, the OCM, CM and IM must be lining the anatomic esophagus rather than stomach because esophageal submucosal glands and their ducts are not present in the stomach. There are two possible explanations for the absence of esophageal gland ducts in the other 86.4% of biopsies with OCM, CM and IM. The first is that these epithelia lined the anatomic stomach where such ducts are normally absent. The second is that 13.6% is a reasonable prevalence of gland ducts in random biopsies of the esophagus considering the random and somewhat sparse distribution of the glands in the esophagus. In a study of esophagectomy specimens, we have shown that the esophageal glands are distributed in a very variable and random manner in the esophagus. Based on this, the finding of gland ducts in 13.6% of esophageal biopsies appears to us to be a reasonable prevalence of gland ducts in random esophageal biopsies. We also examined the histologic features of OCM, CM and IM in biopsies that did and did not contain glands ducts and found these to be identical in terms of the amount of inflammation present and the foveolar and gland architecture. It seems to us to be unlikely that metaplastic OCM, CM and IM of the esophagus exactly reproduces the histologic features of ‘native’ gastric OCM, CM and IM. In contrast, biopsies lined by oxyntic mucosa was never associated with mucosal gland ducts. In a recent study of esophagectomy specimens, Sarbia et al. reported the finding of gland ducts and submucosal glands under OCM and CM and used this finding to characterize these mucosae as located in the esophagus.

Multilayered epithelium in the glandular epithelia in the region of the gastroesophageal junction was absent in oxyntic mucosa and present in OCM, CM and IM. The total number of cases with multilayered epithelium (68 or 14.4%) and its distribution in OCM, CM and IM was remarkably similar to that of gland ducts in these biopsies, suggesting a possible relationship between the two. In their study of multilayered epithelium, Glickman et al. found that this was frequently related to the openings of the gland ducts. The results of the present study support their contention that multilayered
epithelium may arise from cells in the region of the openings of the gland ducts. The presence of multilayered epithelium in OCM, CM and IM provide excellent evidence that these epithelial types line an esophagus that is in a state of flux between squamous and glandular epithelia due to varying levels of damage by gastroesophageal reflux.

We suggest that histologic examination of the region immediately around the proximal limit of the rugal folds represents the only accurate way of distinguishing gastric from esophageal mucosa. In esophagectomy specimens, the submucosal glands act as a histologic criterion to separate esophagus from stomach. In mucosal biopsies, the ducts of these glands and multilayered epithelium are reliable markers of esophageal mucosa. It is important to recognize that the random and variable distribution of these two markers makes only the positive finding reliable. The absence of these markers does not mean that the mucosa is not esophageal.

In mucosal biopsies from this region, it is accepted that oxyntic mucosa characterizes gastric mucosa. The present study supports this fact because gland ducts and multilayered epithelium were never found in oxyntic mucosa. Controversy exists regarding whether OCM, CM and IM found at the junction represent the lining of the anatomic stomach or esophagus. In the present study, 20.8% of biopsies lined by these glandular mucosae are esophageal by the histologic markers of gland ducts or multilayered epithelium. We suggest that these histologic criteria be given higher priority than endoscopic data in ascribing anatomic location to these biopsies. Thus, mucosal biopsies containing gland ducts and multilayered epithelium are esophageal, irrespective of their stated endoscopic location. The recognition of these histologic criteria of esophageal mucosa should be reported when found and can be used to assess the accuracy of the endoscopist’s assessment of the gastroesophageal junction.

The 79.2% of biopsies in the present study that are lined by OCM, CM and IM and lacking the two histologic criteria of esophageal mucosa still remain controversial as to gastric or esophageal location. Our strong belief that they always represent abnormal mucosae derived from reflux-induced transformation of the esophageal squamous epithelium remains to be proven by methods that cannot be applied to mucosal biopsies.

References