Original contribution

Distinguishing Barrett gastric foveolar dysplasia from reactive cardiac mucosa in gastroesophageal reflux disease

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Summary Morphologic dysplasia remains the criterion standard of cancer risk in Barrett esophagus but poses many challenges including distinction from reactive inflammatory change. Gastric foveolar dysplasia, a newly described subtype comprising 15% to 20% of Barrett dysplasia, overlaps with reactive cardiac mucosa in gastroesophageal reflux disease (GERD). Despite the clinical importance of accurate distinction, the issue has not been studied. Review of 3698 biopsies from 461 Barrett patients yielded 160 biopsies with Barrett gastric foveolar dysplasia (74 low grade and 86 high grade). These were compared with inflamed cardia from 80 patients with GERD. Immunohistochemistry was performed for Lgl2, MUC2, MUC5AC, and MUC6. Comparing GERD with Barrett gastric foveolar dysplasia, surface nuclear stratification (85% versus 0%, $P < .00001$), upper mucosa–limited atypia (80% versus 0%, $P < .0001$), villiform architecture (52% versus 4%; $P < .0001$), full-thickness mucosal atypia (0% versus 100%, $P < .00001$), and crowded glandular architecture (0% versus 75%, $P < .00001$) all proved useful. Cytologic features were less helpful. Comparing low-grade gastric dysplasia alone, because its distinction from reactive cardia may be even more challenging, the listed features all remained significant. Loss or aberrant Lgl2 expression was much more typical of dysplasia (12% versus 99%; $P = .0001$). MUC proteins did not distinguish the groups. Surface nuclear stratification, “top-heavy” atypia, and noncrowded, villiform architecture were highly characteristic of reactive cardiac atypia in GERD, in comparison with the monolayered nuclei in crowded glands occupying the full mucosal thickness in Barrett gastric foveolar dysplasia. Loss or aberrant Lgl2 staining was useful in identifying Barrett gastric foveolar dysplasia.

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1. Introduction

Barrett esophagus is a well-established risk factor for esophageal adenocarcinoma [1]. Recognition of dysplasia on endoscopic biopsies remains the criterion-standard cancer risk marker but remains fraught with difficulty. Distinction from reactive inflammatory change in gastroesophageal reflux disease (GERD) is one of the most important problems and forms the focus of this study.
Until recently, only intestinal-type dysplasia was recognized and investigated in Barrett esophagus. The accepted criteria for Barrett dysplasia conformed entirely to the intestinal type, with its nuclear stratification, variable polarity, and wide range of pleomorphism. The second type of Barrett dysplasia, namely, the gastric foveolar type, is now gaining increasing recognition. Its prevalence, diagnostic criteria, and natural history are beginning to emerge [2-4]. Recently proposed criteria for gastric foveolar-type Barrett dysplasia [3] include its uniformly monolayered and basally oriented nuclei with a narrower range of pleomorphism and variable nuclear sizes and nucleoli. Both forms of dysplasia, intestinal and gastric, replace the full thickness of the mucosa, and both exhibit variable glandular crowding.

Much histologic overlap exists between Barrett gastric foveolar dysplasia and reactive, inflamed cardiac mucosa, in the setting of either GERD or Barrett esophagus. The distinction is of obvious clinical importance, considering not only the extremely high prevalence of benign reflux disease versus true dysplasia but also the major management differences. Despite this, the distinction between these 2 epithelia has not been systematically analyzed and forms the basis of the present study.

Lethal giant larva (LGL) is a gene involved in regulating maintenance of epithelial cell polarity and asymmetric cell division. In a recent study, loss or aberrant expression of Lgl2 protein was noted in almost all cases of foveolar epithelial dysplasia and adenocarcinomas of the stomach [5].

Previous studies have also suggested that MUC expression is associated with Barrett neoplastic progression [6-8], but their specific application to Barrett gastric foveolar dysplasia has not been well studied. We therefore explored the expression of Lgl2, MUC2, MUC5AC, and MUC6 as potential adjuncts to morphology.

2. Materials and methods

2.1. Subject selection

After institutional review board approval, Barrett dysplasia cases, as defined by criteria outlined by American College of Gastroenterology [9], were culled from the pathology archives between January 2006 and July 2009. For comparison, biopsies from patients with clinical and endoscopic evidence of GERD with severe reactive inflammatory change were also retrieved.

2.2. Histology

All hematoxylin and eosin (H&E)–stained biopsies were diagnosed by simultaneous review and consensus agreement by 3 gastrointestinal pathologists and a pathology resident (M.P.B., A.E.B., D.T.P., and D.M.). Previously outlined criteria [3] were used to identify 14 additional patients with Barrett gastric foveolar dysplasia. These, in addition to the cohort used in our previous study with gastric foveolar dysplasia [3], were used in the subsequent comparison with reactive cardia. Biopsies from both cohorts were systematically evaluated for (a) architecture: glandular versus villiform, crowded versus noncrowded glands, and extent of mucosal involvement (lack of surface maturation or full-thickness mucosal atypia versus “top-heavy” atypia in which the surface epithelial cells demonstrate more hyperchromatic, atypical, and stratified nuclei compared with the basal glands lined by cells with basally oriented, normochromatic nuclei and abundant mucinous cytoplasm), and (b) cytology: nuclear stratification, nuclear size, pleomorphism, nucleoli, and quality of cytoplasm (mucinous, oncocytic, or eosinophilic).

Gastric foveolar dysplasia was graded using previously described criteria [3]. Briefly, low-grade dysplasia had dysplastic changes involving both the crypts and the surface epithelium (“full-thickness mucosal involvement”). There was minimal glandular crowding, and nuclear enlargement was less than 3 to 4 times the size of a small lymphocyte. High-grade dysplasia also had full-thickness mucosal involvement but more marked architectural crowding and nuclei that were 3 to 4 times or more the size of a small lymphocyte. Because intramucosal adenocarcinoma typically can be readily distinguished from reactive atypia of GERD, these biopsies were excluded from the analysis. All the cases were evaluated for goblet cells.

2.3. Immunohistochemistry

Immunohistochemistry was performed on 78 of 80 biopsies of reactive cardia and 158 of 160 biopsies of Barrett gastric foveolar dysplasia, and the details of antibodies are listed in Table 1. The pattern of Lgl2 expression (basolateral membranous, cytoplasmic, negative) and the intensity (weak, moderate, strong) were scored in a semiquantitative manner [5]. When more than 1 pattern was present, the major pattern (immunoreactivity in >50% cells) and the minor pattern (immunoreactivity in <50% cells) were recorded. Cytoplasmic or apical membrane staining was considered aberrant expression. To allow comparison with published literature, cytoplasmic expression (surface, deep, or full thickness) in 10% or more cells was considered positive staining for MUC proteins.

2.4. Statistical analysis

Dichotomous measures were compared using the χ² test. In addition, differences in histologic variables between the 2

<table>
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<tr>
<th>Table 1</th>
<th>Antibodies for immunohistochemistry</th>
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<tr>
<td>Antigen</td>
<td>Clone</td>
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<tr>
<td>Lgl2</td>
<td>4G2</td>
</tr>
<tr>
<td>MUC2</td>
<td>Cep58</td>
</tr>
<tr>
<td>MUC5AC</td>
<td>CLH2</td>
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<tr>
<td>MUC6</td>
<td>CLH5</td>
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groups were also analyzed. A $P$ value of less than .05 was considered statistically significant.

3. Results

3.1. Clinical and pathologic features

A total of 3698 biopsies from consecutive patients diagnosed as having Barrett esophagus were reviewed from January 2006 to July 2009 to identify 1779 biopsies from 461 patients with Barrett-related dysplasia. A total of 44 (9.5%) of 461 patients and 160 (8.9%) biopsies with Barrett gastric foveolar dysplasia (74 low grade and 86 high grade) formed the cohort of this study. Because both extramural and intramural cases were included in this study to enrich for cases with gastric dysplasia and better address the distinction from GERD and not natural history, these numbers do not reflect true prevalence.

Of the 44 patients with gastric foveolar dysplasia (mean age, 70 years; range, 42-84 years), 25 had pure gastric foveolar dysplasia (no intestinal-type dysplasia) and the remaining 19 cases demonstrated mixtures of biopsies with intestinal and other biopsies with gastric foveolar dysplasia. Only the gastric foveolar dysplasia in each case was further considered for purposes of this study.

The comparison group consisted of 80 patients with GERD and reactive gastric cardiac mucosa without evidence of Barrett esophagus. Their mean age (58 years; range, 25-83 years) was significantly lower than that of subjects with Barrett gastric foveolar dysplasia ($P = .001$). Although dysplasia was more common in men, the difference in male-to-female distribution between the 2 groups (3.5:1 versus 1.7:1) was not significant.

3.2. Comparative analysis

All cases of Barrett gastric foveolar dysplasia demonstrated goblets cells in at least 1 biopsy fragment. In contrast, only a single case of reactive cardiac mucosa showed rare goblet cells (99% versus 1%, $P = .0001$). This patient lacked endoscopic features of Barrett and was thus still classified as GERD according to the American College of Gastroenterology definitions. Considering the assessed architectural features (Fig. 1), compared with reactive cardiac mucosa in GERD, Barrett gastric foveolar dysplasia cases were characterized by full-thickness mucosal atypia (sensitivity 100%, specificity 100%), crowded glands (sensitivity 75%, specificity 100%), and nonvilliform (glandular) growth (sensitivity 96%, specificity 52%). These features were all very useful diagnostically and highly significant ($P < .0001$; Table 2). Biopsies with reactive atypia in GERD more commonly displayed a villiform architecture with top-heavy atypia or surface atypia.

Cytologically, foveolar dysplasia could be distinguished from reactive atypia of cardiac mucosa by lack of nuclear stratification ($P < .0001$, sensitivity 100%, specificity 85%), more common nucleioli ($P < .0001$, sensitivity 81%, specificity 66%), and nuclear enlargement ($P = .0003$, sensitivity 50%, specificity 89%; Table 2). However, these cytologic features, in general, demonstrated lower sensitivities and specificities compared with the architectural features. Although nuclear pleomorphism was more commonly noted in dysplastic epithelium, this difference was of borderline significance (31% versus 10%; $P = .07$) and had poor sensitivity (31%) and good specificity (90%). Most biopsies with reactive cardiac mucosa showed mucinous cytoplasm (95%), with the rest demonstrating eosinophilic cytoplasm (5%). In comparison, 44% biopsies with foveolar dysplasia demonstrated mucinous cytoplasm; the remaining cases showed oncocytic (17%) or eosinophilic cytoplasm (39%).

Because more than half the Barrett gastric foveolar dysplasia cases were categorized as high-grade dysplasia, we assessed the significance of the grade of foveolar dysplasia in distinguishing reactive cardia and dysplasia (Table 2). Irrespective of the dysplasia grade, the architectural features continued to prove helpful. Cytologically, there was a significant overlap in the degree of nuclear pleomorphism and nuclear size between low-grade gastric foveolar dysplasia and GERD, and although the specificity rates for these features were identical to combined low-grade and high-grade gastric foveolar dysplasia, the sensitivity decreased from 50% and 31% to 12% and 20%, respectively. This was, however, not the case for the high-grade dysplasia cohort, where nuclear pleomorphism and nuclear enlargement were significantly associated with the dysplasia category.

3.3. Lgl2 immunohistochemistry

Compared with reactive cardiac epithelium, wherein 69 (88%) of 78 biopsies showed basolateral membranous Lgl2 staining, only 1 (1%) of 158 cases of foveolar Barrett dysplasia showed basolateral membranous expression as the major pattern within the surface epithelium ($P = .0001$; Fig. 2). Among the 69 biopsies of reactive cardiac mucosa with basolateral staining as the major pattern, the distribution of intensity of expression was strong (34 [50%]), moderate (30 [43%]), and weak (5 [7%]). Absence of staining within the surface epithelium was the major pattern in 139 (88%) of 158 cases of foveolar dysplasia.

Aberrant expression in the form of apical and/or cytoplasmic staining was noted in both cohorts. Apical staining was seen only in biopsies with foveolar dysplasia (12/157; 8%), whereas weak cytoplasmic expression was seen in 6 (4%) of 157 biopsies of Barrett gastric foveolar dysplasia and in 6 (8%) of 75 biopsies of reactive cardiac mucosa. Loss or aberrant expression Lgl2 expression thus had an overall sensitivity of 99% and specificity of 88% for a diagnosis of Barrett gastric foveolar dysplasia.
Barrett gastric foveolar dysplasia versus reactive cardia

Fig. 1  A, Reactive gastric cardiac mucosa in GERD showing top-heavy atypia with mucin loss and nuclear hyperchromasia of the glands in the top half of the mucosa, along with a villiform, noncrowded architecture (H&E, ×40). B, Higher magnification demonstrating surface nuclear stratification with prominent active inflammation (×200). C, Barrett gastric foveolar dysplasia showing full-thickness mucosal replacement by crowded glands and nonvilliform architecture (×40). D, Barrett gastric foveolar low-grade dysplasia composed of cells with basally oriented monolayered and uniform nuclei with abundant pale eosinophilic to mucinous cytoplasm. Also present is a focus of intestinal metaplasia (lower right; ×200). E and F, Barrett gastric foveolar high-grade dysplasia with crowded glandular architecture and marked nuclear enlargement of 3 to 4 times or more the size of an adjacent small mature lymphocyte nucleus (E: ×200, F: ×400).
MUC2, MUC5AC, and MUC6 immunohistochemistry

MUC2 immunoreactivity was present predominantly in the goblet cells dispersed within the associated intestinalized mucosa. The dysplastic gastric foveolar epithelium and the reactive cardiac were both essentially negative (0% versus 2%, \( P = .79 \); Fig. 3A, B). Surface epithelial cytoplasmic MUC5AC expression and nonreactive deep glands were observed in all 78 (100%) of 78 biopsies of reactive cardia and 143 (92%) of 155 biopsies of gastric foveolar dysplasia (Fig. 3 C, D). MUC6 staining was complementary to MUC5AC; only the deep glands in both the groups showed strong cytoplasmic expression, whereas the superficial epithelium was completely negative, without differences between dysplasia and GERD (Fig. 3E, F). Thus, MUC2, MUC5AC, and MUC6 staining had no use in distinguishing between reactive cardia and gastric foveolar-type Barrett dysplasia.

4. Discussion

Despite some of the challenges in diagnosing dysplasia [10,11], its identification on routinely processed endoscopic biopsies remains the criterion standard of cancer risk in Barrett esophagus. Inflammatory and reactive/regenerative changes, so commonplace in GERD, are among the most serious of the diagnostic challenges. Because foveolar epithelium is a component of the mosaic epithelium that comprises Barrett mucosa, it is not surprising that this cell type, too, may develop neoplastic change that needs to be differentiated from benign, inflamed cardiac mucosa in GERD. We address this problem in Barrett neoplasia relative to this newcomer in its diagnostic classification, Barrett gastric foveolar dysplasia. This subtype has a distinctive and usually more subtle morphology that does not lend itself to the standard intestinal-type diagnostic and grading criteria for Barrett dysplasia. This study now provides useful and practical morphologic criteria, along with an immunophenotypic adjunct, to help distinguish these 2 important look-alikes. The critical management differences between dysplasia and GERD emphasize the importance of this distinction.

<table>
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<tr>
<th>Table 2</th>
<th>Morphologic comparison of Barrett gastric foveolar dysplasia and reactive cardia</th>
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<tbody>
<tr>
<td><strong>Histologic feature</strong></td>
<td><strong>Reactive gastric cardia (n = 80)</strong></td>
</tr>
<tr>
<td><strong>Architectural features</strong></td>
<td></td>
</tr>
<tr>
<td>Atypia</td>
<td>80 (100%)</td>
</tr>
<tr>
<td>Full-thickness</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Glandular crowding</td>
<td>80 (100%)</td>
</tr>
<tr>
<td>Crowded</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Glandular configuration</td>
<td>42 (52%)</td>
</tr>
<tr>
<td>Glandular</td>
<td>38 (48%)</td>
</tr>
<tr>
<td><strong>Cytologic features</strong></td>
<td></td>
</tr>
<tr>
<td>Nuclear stratification</td>
<td>14 (15%)</td>
</tr>
<tr>
<td>Stratified</td>
<td>68 (85%)</td>
</tr>
<tr>
<td>Nucleoli</td>
<td>53 (66%)</td>
</tr>
<tr>
<td>Present</td>
<td>27 (34%)</td>
</tr>
<tr>
<td>Nuclear pleomorphism</td>
<td>72 (90%)</td>
</tr>
<tr>
<td>Pleomorphic</td>
<td>8 (10%)</td>
</tr>
<tr>
<td>Nuclear size</td>
<td></td>
</tr>
<tr>
<td>&lt;3-4 times small lymphocyte</td>
<td>71 (89%)</td>
</tr>
<tr>
<td>≥3-4 times small lymphocyte</td>
<td>9 (11%)</td>
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</tbody>
</table>

Abbreviations: BGFD, Barrett’s gastric foveolar dysplasia; LG, low grade; HG, high grade.
NOTE. \( P \) values are for BGFD compared to reactive cardia.
gastric foveolar Barrett dysplasia. Thus, by inference, these earlier studies were based in most intestinal-type Barrett dysplasia. Accordingly, the villiform change reported in these earlier studies does not readily apply to our study’s finding that villiform change in gastric cardiac-type mucosa is highly indicative of nonneoplastic GERD rather than Barrett gastric foveolar dysplasia. It appears that the significance of villiform change must be carefully considered relative to whether intestinal or gastric foveolar mucosa is being evaluated, but further study of this issue will be necessary.

Among the cytologic features, lack of nuclear stratification ($P < .0001$), prominent nucleoli ($P < .0001$), and nuclear enlargement ($\geq 3$-4 times the size of a mature lymphocyte; $P = .0003$) were more commonly seen in Barrett gastric foveolar dysplasia. Nuclear pleomorphism demonstrated a trend toward significant association with Barrett gastric foveolar dysplasia compared with reactive cardia ($P = .07$), but this was largely due to the high-grade dysplasia subset where it was significant ($P = .003$).

Because 53% of cases of Barrett gastric foveolar dysplasia in this series were high-grade dysplasia, a subgroup analysis of low- and high-grade dysplasia was performed. It is reasonably argued that high-grade dysplasia may be more easily distinguished from benign cardia than low-grade dysplasia and that the inclusion of high-grade dysplasia could well bias the results. Fortunately, this was not the case for most criteria.

Surface-predominant or top-heavy atypia with relative sparing of the deeper glands in reactive gastric cardia is likely a reflection of the location of the stem cell/regenerative compartment within gastric mucosa. This resides in the midportion of the gastric mucosa or the gastric neck region, compared with the basal crypt location in intestinal mucosa. Reactive/regenerative change therefore begins midway within the mucosa and extends onto the surface epithelium, creating the top-heavy atypia distribution pattern. In contrast, the greatest atypia in intestinal epithelium resides in the basal-most glands creating a bottom-heavy atypia pattern. In contrast, dysplasia almost always shows full-thickness involvement of the mucosa, whether gastric or intestinal type. Full-thickness mucosal involvement was consistently present in the gastric foveolar dysplasia cases in our study, and for this reason, in fact, full-thickness involvement is a major diagnostic criterion for dysplasia.

A recent study demonstrated loss or aberrant expression of Lgl2 in foveolar dysplasia and adenocarcinoma of the stomach, relative to retention in reactive gastric mucosa [5]. Our study confirms these findings in Barrett gastric foveolar dysplasia, wherein most cases showed loss or aberrant cytoplasmic/apical Lgl2 expression. The surface foveolar epithelium of reactive gastric cardia in GERD showed intact moderate to strong basolateral expression of this cell polarity protein, consistent with its proposed function of maintaining cellular framework [13,14]. Differential expression of Lgl2 was therefore confirmed as a useful adjunct to H&E morphology for distinguishing benign cardia in GERD from Barrett foveolar dysplasia. As previously reported in gastric mucosa [5], we also found that Lgl2 expression was consistently absent in foci of intestinal metaplasia admixed with separate areas of Barrett gastric foveolar dysplasia.

Alteration of normal MUC patterns in diseases such as inflammatory bowel disease, Barrett esophagus, chronic gastritis, and gastric neoplasia [7] has led to their study as potential biomarkers of disease progression, most notably Barrett-related neoplastic progression [6,8,15,16]. However, these Barrett studies also predate the distinction between intestinal- and gastric-type dysplasia in Barrett esophagus, prompting further study here. Of the MUC2, MUC6, and MUC5AC panel performed in this study, none showed the use of the distinction between reactive cardia in GERD and Barrett gastric foveolar dysplasia.
In summary, Barrett gastric foveolar dysplasia is a subtype of Barrett dysplasia that can be differentiated from reactive/regenerative cardiac atypia of GERD using practical H&E morphology with adjunctive Lgl2 immunohistochemistry as needed in difficult cases. MUC protein immunoreactivity was not helpful and acted as only surrogate markers.
of intestinal metaplasia or gastric cardiac-type mucosa in general, features readily identifiable on H&E alone. The most useful morphologic criteria from this systematic review include, in GERD, the following: surface nuclear stratification (sensitivity 85%, specificity 100%), top-heavy mucosal atypia (sensitivity 100%, specificity 100%), noncrowded glands (sensitivity 100%, specificity 75%), and villiform architecture (sensitivity 52%, specificity 96%). Alternatively, in gastric foveolar Barrett dysplasia, loss or aberrant expression of Lgl2 (sensitivity 99%, specificity 88%), full-thickness mucosal involvement (sensitivity 100%, specificity 100%), and glandular crowding (sensitivity 96%, specificity 52%) were useful. Application of these criteria should significantly improve the distinction between the look-alikes of gastric foveolar dysplasia and reactive cardia in GERD.

References


[16] Warson C, Van De Bovenkamp JH, Korteland-Van Male AM, et al. Barrett’s esophagus is characterized by expression of gastric-type mucins (MUC5AC, MUC6) and TFF peptides (TFF1 and TFF2), but the risk of carcinoma development may be indicated by the intestinal-type mucin, MUC2. Hum Pathol 2002;33:660-8.