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Original contribution

Intestinal metaplasia around the gastroesophageal junction is frequently associated with antral reactive gastropathy: implications for carcinoma at the gastroesophageal junction☆,☆☆

Monika Vyas MBBS [a], Romulo Celli MD [a], Manpreet Singh MD [b], Natalie Patel MD [a], Harry R. Aslanian MD [b], Daniel Boffa MD [c], Yanhong Deng MPH [d], Maria M. Ciarleglio PhD [d], Loren Laine MD [b,e], Dhanpat Jain MD [a,*]

[a] *Department of Surgical Pathology, Yale University School of Medicine, New Haven, CT, USA*
[b] *Department of Internal Medicine (Section of Digestive Diseases), Yale University School of Medicine, New Haven, CT, USA*
[c] *Surgery, Yale School of Medicine, New Haven, CT, USA*
[d] *Yale Center for Analytical Sciences, Yale University School of Public Health, New Haven, CT, USA*
[e] *Department of Internal Medicine (Section of Digestive Diseases), VA-CT Healthcare System, West Haven, CT, USA*

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Summary Increasing evidence suggests that bile reflux (BR) plays a major role in mucosal injury, leading to adenocarcinoma of the proximal stomach and distal esophagus. However, gastric BR is difficult to diagnose and investigate. Reactive gastropathy (RG), in the absence of nonsteroidal anti-inflammatory drugs (NSAIDs) and other known causes, likely represents bile-mediated injury to the gastric mucosa. The goal of this study is to explore the association between antral RG and gastroesophageal junction (GEJ) mucosal inflammation and intestinal metaplasia (IM). The pathology database was searched for patients who had gastric biopsies with a diagnosis of antral RG and concurrent gastric cardia/GEJ/distal esophagus biopsies from 2013 to 2015. Age- and sex-matched patients with normal gastric antral biopsies served as controls. Biopsies from the GEJ region were evaluated for histological changes, including inflammation, antral and pancreatic metaplasia, RG, the type of gastric glands,

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* Corresponding author. Department of Pathology, Yale School of Medicine, 310 Cedar St, New Haven, CT, 06520, USA.
E-mail address: dhanpat.jain@yale.edu (D. Jain).

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proton pump inhibitor (PPI) changes, and IM. Detailed clinical history and medication use (including PPIs and NSAIDs) were recorded. IM in the GEJ region was more frequent in patients with antral RG than in controls (33.0% vs. 5.2%, 95% confidence interval [18.3−37.3%]). In addition, inflammation, other mucosal changes around the GEJ (RG and foveolar hyperplasia), antral IM, and PPI-associated mucosal changes were also more frequently seen in patients with antral RG. Our results show that antral RG is associated with mucosal injury and IM around GEJ, suggesting a role of BR. Further studies are needed to study duodenogastric-esophageal BR and its role in development of proximal gastric and distal esophageal adenocarcinoma.

1. Introduction

Bile reflux (BR) is believed to play an important role in the induction of gastric mucosal injury, leading to carcinoma in the region around the gastroesophageal junction (GEJ). In vitro studies and animal experiments show that bile is far more potent than acid in inducing intestinal metaplasia (IM)/Barrett's esophagus (BE) and cancer [1,2]. Bile can result in inflammation, IM, and cancer in both the gastric and esophageal squamous mucosa, ie, the mucosa above and below the GEJ. However, it is difficult to diagnose BR, and no effective therapies are currently available for treatment of BR.

Reactive gastropathy (RG), in the absence of nonsteroidal anti-inflammatory drug (NSAID) use and other known causes (chemical irritants such as alkaline agents or alcohol, oral iron supplements, or other medications), has been thought to represent bile-mediated gastric mucosal injury in most cases [3]. One can postulate that if bile is indeed involved in the development of IM and adenocarcinoma around the GEJ (proximal stomach and distal esophagus [DE]), the incidence of inflammation, IM, and dysplasia around the GEJ should be higher in patients who show bile-induced RG changes in the gastric antrum. If this is true then, bile-induced RG may be a marker for future risk of cancer around the GEJ. With this goal in mind, we reviewed the biopsies around the GEJ (gastric cardia, GEJ, and DE) for IM in patients with a diagnosis of antral RG and compared them with those in patients with normal antral histology.

The goal of this study was to explore the association between antral RG and a variety of histological changes in the gastric cardia/GEJ/DE that include IM, inflammation in the squamous and columnar mucosa, and mucosal changes in the columnar mucosa (foveolar hyperplasia, antral and/or pancreatic metaplasia, and dysplasia).

2. Materials and methods

The pathology database between 2013− and 2015 was searched for patients who had gastric antral biopsies and concurrent mucosal biopsies around the GEJ. For the purpose of the study, biopsies from the *around the GEJ* included specimens that were labeled as gastric cardia, GEJ, or DE. The slides were reviewed, and patients with antral RG were identified (n = 115), which constituted the study group. The histologic features of RG include (1) reactive foveolar hyperplasia with a corkscrew appearance, (2) mucin depletion of the foveolar epithelium, (3) nuclear changes in the foveolar epithelium consisting of smudgy or hyperchromatic nuclei and increased mitosis, and (4) lamina propria inflammation being no more than minimal to mild. These changes were present in all cases of RG in variable amount and degree. For grading the RG, foveolar hyperplasia, lamina propria vascular congestion, and smooth muscle hyperplasia were subjectively graded as mild, moderate, and severe and given scores of 1, 2, and 3, respectively, as per the scheme proposed by Wolf et al. [4] (Fig. 1 A−D). The scores were added to get a final score. A cutoff value of 4 was used for the diagnosis of RG, and RG was graded as mild (<6), moderate (6−7), and severe (>7). The hematoxylin and eosin−stained slides of the biopsies were blindly reviewed by two pathologists (M.V. and D.J.) together, and a consensus score was used. Patients with known history of NSAID use or known significant alcohol intake were excluded from the study group. An equal number of patients (n = 115) during the same study period with normal gastric antral biopsies and concurrent biopsies around the GEJ were included as controls. Clinical presentation and endoscopic findings were recorded for each case. The biopsies from around the GEJ were evaluated for nature and grade of inflammation in both the squamous and columnar mucosa, RG, the presence of *Helicobacter pylori* infection, foveolar hyperplasia, pyloric and pancreatic metaplasia, proton pump inhibitor (PPI) changes, and IM. Presence of inflammatory changes was evaluated in the squamous mucosa when present and was considered reflux-type when two or more of the following features were present: (1) basal cell hyperplasia, (2) rete peg elongation, (3) intraepithelial inflammatory cells, and (4) intracellular edema. The inflammatory changes in the columnar mucosa were subjectively graded as mild, moderate, or severe. The PPI-induced changes in the oxyntic glands included the combination of (1) glandular dilatation, (2) luminal

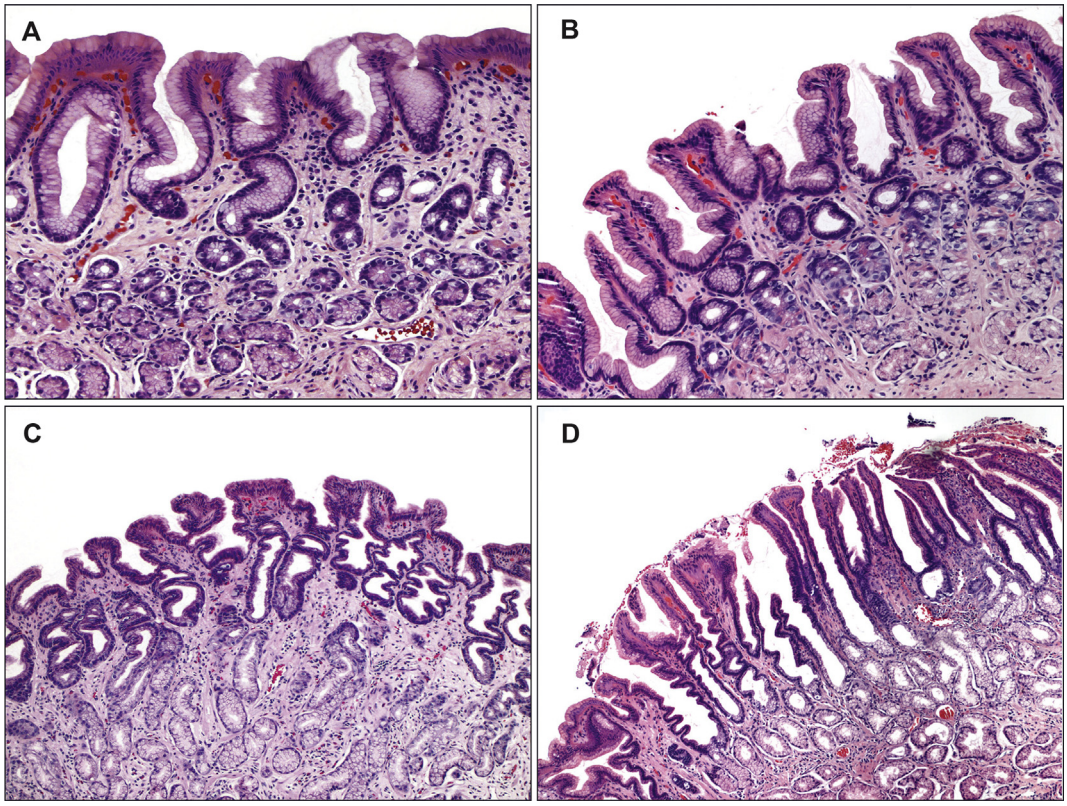

Fig. 1 A, Normal antrum (H&E, ×100). B–D, Gastric antral mucosa showing reactive gastropathy (1B – grade 1+, 1C – grade 2+, 1D – grade 3+) (H&E, ×100). H&E, hematoxylin and eosin.

protrusion of parietal cells, and (3) apical cytoplasmic snouts in the parietal cells. The nature of the gastric glands in the biopsies around the GEJ was recorded as oxyntic, cardio-oxyntic (oxyntic glands mixed with antral-type mucus glands), or antral-type. The antral biopsies were also evaluated for IM and *H. pylori* infection. Patients with concurrent *H. pylori* infection were excluded from the study group. Whenever biopsies from the gastric body or fundus were available, they were reviewed as well. In addition, clinical history including presenting symptoms, prior biopsy results, history of BE/IM around the GEJ, intake of PPIs, and NSAID or other drug use was noted from electronic medical records (EMRs). The endoscopic impression (salmon-colored mucosa, irregular Z-line, erythema erosions, or ulceration) was also recorded. Approval was obtained from the Institutional Review Board at the Yale School of Medicine (see Fig. 2).

2.1. Statistical methods

Demographic and baseline characteristics were compared between groups using Student's t-test for continuous variables and the chi-square test or Fisher's exact test for categorical variables. Continuous variables were summarized as mean and standard deviation, and categorical variables were summarized as count and percentage. The clinical outcome of IM at the GEJ was modeled using multivariate logistic regression. Stepwise model selection was used to help arrive at the final parsimonious model. *P*-values less than or equal to 0.05 were considered statistically significant. All analyses were carried out using SAS (version 9.4; Cary, NC).

3. Results

3.1. Clinical findings

Table 1 summarizes the baseline characteristics for the RG (test) and control groups. The patients in the RG group were slightly older (57.7 vs.51.9 years), whereas there was no significant difference in gender distribution. More patients in the RG group (14.7%) had a prior clinical history of BE recorded in the medical chart than the control group (2.6%) (Table 1).

3.2. Endoscopic findings

More patients in the RG group (18.2%) had a concurrent endoscopy, which is suggestive of BE (salmon patch), than controls (6.0%). Patients in the RG group had more often gastric findings (gastritis/erythema/erosions) than those in

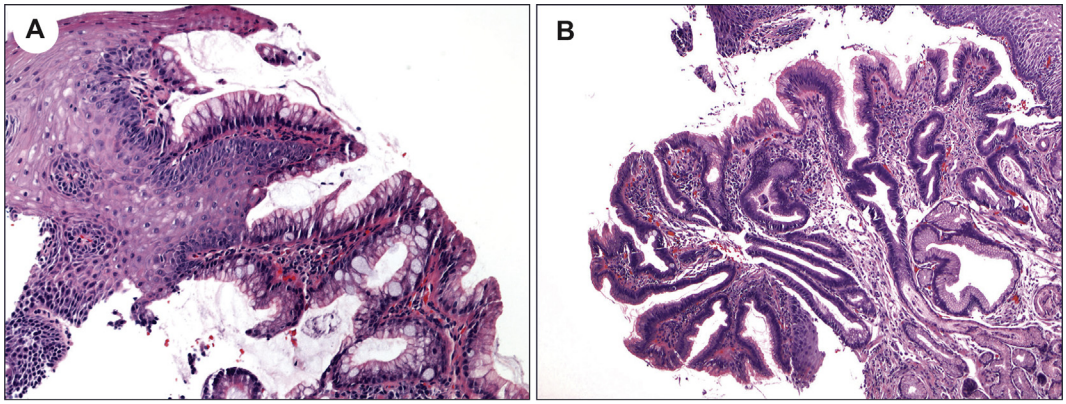

Fig. 2 A, Intestinal metaplasia involving the junctional columnar mucosa in a case with antral reactive gastropathy (H&E, ×200). B, Columnar mucosa at the gastroesophageal junction showing foveolar hyperplasia and reactive gastropathy−type changes (H&E, ×100). H&E, hematoxylin and eosin.

the control group, whereas erosive esophagitis erosions were less frequent (Table 1).

3.3. Histologic findings

During the study period of 2 years, a total of 1257 patients had biopsies from the area around the GEJ, which included 762 biopsies from the GEJ, 546 from the DE, and 39 from the gastric cardia. As per the selection criteria, the study group comprised patients who had RG in the gastric antral biopsies and also had biopsies obtained from the area around the GEJ. In the study group, the biopsy sites from the area around the GEJ were recoded as GEJ (n = 107), DE (n = 8), and cardia (n = 0) compared with GEJ (n = 95), DE (n = 14), and cardia (n = 6) in controls. For the purpose of further analysis, biopsies from the DE, GEJ, and gastric cardia were all lumped together. Table 2 compares the histologic findings between the RG (test) and control groups. The junctional columnar/gastric mucosa was not available for evaluation in 11 of test and 9 of control patients. No squamous mucosa was available for review in 5 of test and 9 of control patients. Overall, more patients in the RG group showed IM around the GEJ, inflammatory changes in the distal esophageal squamous or columnar mucosa, IM in the gastric antrum, and foveolar hyperplasia and RG in the columnar mucosa around the GEJ (Fig. 2), and the findings were statistically significant as per univariate analysis (Table 2). Histologic changes suggestive of treatment with PPIs in the oxyntic mucosa, either in the gastric cardia or corpus biopsies, were also more commonly seen in the patients in the RG group than those in the control group. Based on the results of multivariate stepwise logistic regression, the only findings that were independently associated with IM around the GEJ included antral RG and inflammation around the GEJ (squamous and columnar mucosa) (Table 3).

In 31 of 38 (81.5%) patients with IM, this was the first histologic diagnosis of IM in the biopsies around the GEJ. Of the remaining 7 patients with prior biopsies that showed IM, only 2 had prior biopsies from the gastric antrum, which were reported as normal (slides were not available for review).

Table 1 Baseline characteristics in the study and control group.

Baseline characteristics	Study group (n = 115)	Control (n = 115)	*P*-value
Average age in years (SD)	57.75 (12.28)	51.90 (13.98)	<0.001
Sex (females/total, %)	48/115 (41.74%)	59/115 (51.30%)	0.15
Clinical history: reflux	51/115 (44.35%)	55/115 (47.83%)	0.60
Abdominal pain	15/115 (13.04%)	18/115 (15.65%)	0.68
Nausea/vomiting	10/115 (8.70%)	5/115 (4.35%)	0.25
Anemia	9/115 (7.83%)	12/115 (10.43%)	0.80
History of Barrett's esophagus	17/115 (14.78%)	3/115 (2.61%)	0.001
PPI use	64/71 (90.14%)	17/47 (36.17%)	<0.001
Endoscopic findings: salmon patch	21/115 (18.26%)	7/101 (6.93%)	0.015
Irregular Z-line	56/115 (48.70%)	47/115 (40.87%)	0.32
Erosive esophagitis	17/115 (14.78%)	59/115 (51.30%)	<0.001
Gastritis/erythema/erosions	54/115 (46.9%)	34/115 (29.56%)	0.019

Abbreviations: SD, standard deviation; PPI, proton pump inhibitor.

Table 2 Pathologic features in the study and control groups.

Pathologic features	Study group (n = 115)	Control group (n = 115)	*P*-value
IM at GEJ/cardia	38/115 (33.04%)	6/115 (5.22%)	<0.001
Low-/high-grade dysplasia GEJ	2/115 (1.74%)	0/115	<0.001
Antral IM	12/115 (10.43%)	0/115	<0.001
Moderate to severe carditis (grade 2–4)	26/113 (23.01%)	8/109 (7.34%)	0.001
PPI changes in the body/fundus	57/115 (49.57%)	19/109 (17.43%)	<0.001
Reactive gastropathy–type changes in the cardia	9/104 (8.65%)	1/106 (0.94%)	0.009
Foveolar hyperplastic changes in the cardia	46/110 (41.82%)	5/115 (4.35%)	<0.001
Reflux-type changes in the squamous epithelium	61/110 (55.45%)	25/64 (39.06%)	0.037
Pancreatic metaplasia	7/105 (6.67%)	7/115 (6.09%)	0.86

Abbreviations: IM, intestinal metaplasia; GEJ, gastroesophageal junction; PPI, proton pump inhibitor.

Most patients had grade 1 antral RG (53%), whereas 39% had grade 2 and 8% had grade 3 antral RG. Among those with grade 1 antral RG, 26% cases had IM around the GEJ, whereas 6% of those with grade 2 antral RG and none of those with grade 3 antral RG had IM around the GEJ. The incidence of IM in the antrum was similar in the 3 categories. Presence of IM around the GEJ and other histologic findings failed to show any correlation with the increasing grade of antral RG.

4. Discussion

The incidence of adenocarcinoma of the DE, GEJ, and gastric cardia has been increasing in the Western world, including the United States, in the last few decades and is among the most rapidly rising cancers in Caucasian men, although there is suggestion that it is plateauing [5,6]. This increase has occurred despite the use of acid suppression therapy for gastroesophageal reflux disease (GERD) and increased surveillance of BE [5,6]. The increase has partially been attributed to increasing obesity leading to increasing GERD with resultant BE [7]. The definitions of BE and location of the cancer (DE vs. proximal stomach) have changed over the years and remain a source of controversy. Acid reflux has been considered the main etiologic factor responsible for the BE-related adenocarcinoma, although BR has been increasingly implicated in this regard [8]. There is no evidence that acid suppression decreases the incidence of BE or associated adenocarcinoma, and some have even suggested that PPIs may have just the opposite effect [9–11]. Our results in this regard are interesting as they suggest that patients with RG have higher incidences of chronic inflammation and IM around the GEJ, which supports BR as a potential etiology for the premalignant changes around the GEJ.

Our results show that patients with RG had much higher chances of having IM around the GEJ and a clinical diagnosis of BE than controls. In addition, RG was also strongly associated with other findings suggesting chronic mucosal injury, that included IM in the gastric antrum, inflammation around the GEJ (squamous and/or columnar mucosa), reactive foveolar hyperplasia, and RG-type changes in the columnar mucosa around the GEJ (Fig. 2). In addition, of note, of the 38 patients diagnosed with IM in the study group, this was the initial diagnosis of IM in 31 patients. The findings suggest that BR could be responsible for chronic inflammation and metaplasia in the mucosa around the GEJ. Two patients in the study group also had dysplasia in the columnar mucosa compared with none in the controls, but the numbers are too small to draw any conclusion. We did not find any association between the grades of RG and IM and other inflammatory changes around the GEJ [4]. However, there is also no evidence that histologic scoring for RG has any association with severity of BR. The exact reason for this finding is unclear, but sampling error or the small sample size could be possible reasons.

Another interesting finding in our study is the association of histologic changes associated with chronic PPI use and RG. The clinical history of PPI use as per the EMR was somewhat similar in patients with RG (58.1%) and the control group (41.1%) as many patients in each group had

Table 3 Multivariate logistic regression model of IM at the GEJ.

Feature	OR (95% CI)	Chi-square test statistic	*P*-value
Age	1.05 (1.01–1.10)	5.72	0.017
Sex	3.00 (1.12–8.00)	4.79	0.029
Inflammation at the GEJ (glandular portion)	0.15 (0.04–0.58)	7.57	0.006
Inflammation in the squamous mucosa	4.98 (1.77–13.98)	9.29	0.002
RG in the antrum	8.48 (1.80–39.91)	7.32	0.007

NOTE. Intercept not shown.
Abbreviations: OR, odds ratio; CI, confidence interval; IM, intestinal metaplasia; GEJ, gastroesophageal junction; RG, reactive gastropathy.

GERD symptoms (Table 1); however, the information about the duration of PPI use was not available. On the other hand, the number of cases with mucosal changes associated with PPI use was significantly higher (49.5% vs. 19.1%) in the RG group than in the control group, implying PPIs were used for a longer period of time either owing to longer duration of disease or more severity. Another possible explanation is that bile can be more toxic in the absence of neutralizing effect of gastric acid. Physiologically, it is well recognized that one of the major roles of proximal duodenal secretions is to neutralize acidic contents coming from the stomach. The impact of duodenal reflux in an acid-poor milieu in the stomach remains poorly studied, although duodenal reflux on its own has been shown to be toxic to the gastric mucosa [1,11]. It has been shown that although some patients show reduction in BR in response to PPI therapy, a considerable number of patients who become asymptomatic after initiation of therapy may have persistent BR [12,13].

The interesting feature of our study is the novel design wherein we have used RG as a potential marker of BR and its association with mucosal changes around the GEJ. Rugge et al. [14] studied the gastric mucosal histology in patients with BE and found reduced incidence of *H. pylori* and multifocal atrophic gastritis in these patients; in this study, it appears they did not specifically look for RG. Dixon et al. [15] have also found an association between bile acid exposure, chemical gastropathy, and increased gastric IM, which is similar to our findings of increased incidence of antral IM in patients with RG. However, they did not find a significant difference between the incidence of RG in patients with BE and GERD [16]. They also devised a bile reflux index (BRI) taking into account the degree of RG, IM, and inflammation and showed a higher BRI was associated with increased incidence of BE, although agreement on BRI scoring itself was poor [15]. In a study of gastric biopsies with IM, Genta and Sonnenberg [16] found concurrent RG changes only in about one-fifth of cases; however, majority of the cases showed *H. pylori* and its sequelae. To best of our knowledge, to date, there are no studies comparing the incidence of BE in patients with and without RG changes in the antrum. The implications of these findings are manyfold. First and foremost, it supports the role of BR in carcinogenesis around the GEJ. Our study also raises concern with regard to the role of acid suppression in these patients, wherein lack of acid in the stomach may accentuate mucosal injury due to duodenal BR.

Bile has been known to be a very potent carcinogen in the gastrointestinal (GI) tract and has been implicated in development of BE and gastric adenocarcinoma [17]. Studies using Bilitec apparatus have shown that BR is very common and intragastric bile concentrations are higher in patients with BE than in patients with uncomplicated GERD [13,18]. Sun et al. [1] have demonstrated increased incidence of BE and dysplasia in rats with surgically induced bile acid reflux. They also tested their results by feeding animals separately with deoxycholic acid and acidic water and found increased Barrett-like metaplasia in rats fed with bile acid−treated water, but not with acid alone [1]. Follow-up studies in patients undergoing esophagectomy and gastric pull-through for achalasia have shown increased incidence of BE and adenocarcinoma in postoperative patients, and duodenogastric reflux in addition to acid reflux is implicated in the same patients [19,20]. The unconjugated bile acids have been found to be particularly damaging at neutral pH, which is the case in PPI-treated patients [21]. Again, unconjugated bile acids, such as deoxycholic acid, predominate in patients treated with PPIs, whereas conjugated bile acids are the major component in untreated individuals [21]. Some animal and human studies have demonstrated that bile acids are most toxic when in combination with acids [22]. Unconjugated bile acids and taurine conjugates are more soluble and gain easy entry into the mucosal cells and can enhance IM [23,24]. Acidified bile has also been shown to induce the upregulation of the c-myc oncogene with malignant progression of BE [25]. Still, in humans, the relative roles of bile and acid in inducing neoplasia around the GEJ remain unclear, and more studies are required to analyze their roles.

One of the limitations of the study is that it presumes that all RG cases in the absence of documented use of NSAIDs are indicative of BR. The study relies on EMRs for clinical history, alcohol use, and medications, which may not be completely accurate. The role of NSAIDs and other over-the-counter drugs cannot be completely ruled out in every case. Other factors such as food habits (especially the role of spices) leading to RG are also very difficult to evaluate in such a retrospective study. However, despite these limitations, it is speculated that BR is the etiology of RG in the majority of cases. IM above and below the GEJ remains a subject of controversy, and especially, IM at or below the GEJ has been attributed to many etiologies. We tried to exclude *H. pylori* (HP) as one of the etiologies to the best of our ability in the study cases. Our study being retrospective, suffers from some other obvious flaws. The temporal relationship between the occurrence of BR and IM/BE could not be established. The time point of initiation of PPI therapy cannot be determined accurately, and its impact on BR cannot be studied. It is entirely possible that patients had acid reflux to start with, but the RG developed later, possibly secondary to long-term acid suppression. The inflammation around the GEJ that appears significantly associated with IM was subjectively evaluated and needs better characterization and grading for providing any further insights into the mechanism. Further studies with larger cohorts and more detailed clinical data are required to answer these questions.

In conclusion, our results show that IM is more frequently seen in the DE/GEJ/cardia in patients who have

antral RG than in controls, supporting the notion that BR may play a role in the development of mucosal injury around the GEJ. Our results also show that PPI-associated changes were also significantly associated with antral RG, supporting the idea that PPI use may augment BR by decreasing neutralization by gastric acid. Further studies are needed to study duodenogastric-esophageal BR and its role in development of IM and adenocarcinoma around the GEJ (gastric cardia and DE). More long-term follow-up studies are needed in this area to establish definitive associations. Our study also argues for developing better clinical tests for evaluation of BR and its treatment.

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