# *Helicobacter pylori* infection over bile reflux: No influence on the severity of endoscopic or premalignant gastric lesion development

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**Abstract.** *Helicobacter (H.) pylori* infection and duodeno-gastric reflux (DGR) are both linked to endoscopic and premalignant gastric lesion development, but it is still unclear whether they are independent or have a causal relationship. This study investigated the histologic gastric changes in patients with primary DGR and *H. pylori* infection, as well as their endoscopic findings, symptoms, drug consumption, and social behavior in comparison with patients presenting only DGR. The study included 560 patients with primary DGR on endoscopy divided into two groups, according to the presence/absence of *H. pylori* infection on biopsy (utilizing usual stainings and immunohistochemical methods). There was no significant difference in terms of age and sex, nor in the frequency of diabetes or esophagitis between the studied groups. Epigastric pain was associated with *H. pylori*-positive biopsies in multivariate logistic regression analysis (P=0.005). Although without statistical significance, severe endoscopic lesions and premalignant gastric lesions were more frequent in the *H. pylori* group (45.1 vs. 28.4% and 37.4 vs. 32.3%, respectively). In patients with DGR, the final multivariate model revealed a positive association between smoking and immunohistochemically confirmed *H. pylori* infection (P=0.02, OR=1.88, 95% confidence intervals (CI)=1.10 to 3.21), but a negative effect of proton pump inhibitor consumption (P<0.001, OR=0.50, 95% CI=0.35 to 0.73). In conclusion, in patients with *H. pylori* infection and DGR, epigastric pain was the main reason for the endoscopic investigation. *H. pylori* infection over DGR

did not influence the severity of endoscopic or premalignant gastric lesion development. Furthermore, smoking is directly related to immunohistochemically assessed active *H. pylori* infection in patients with bile reflux.

## Introduction

Duodenogastric reflux (DGR, bile reflux) consists of a retrograde passage of alkaline duodenal and pancreatic content into the stomach, which can lead to mucous barrier disruption and direct chemical damage to the surface gastric epithelium. DGR is common after gastric surgery, cholecystectomy and pyloroplasty, but it can also occur due to antroduodenal motility disorder (primary DGR) (1,2). Primary bile reflux does not have a standardized diagnosis. If an upper digestive endoscopy (UDE) reveals a large amount of bile in the stomach, mucosal fragility, erythema/erosions/ulcers in a patient with epigastric pain, nausea/vomiting, the diagnosis is usually DGR (3).

Gastric cancer (GC), particularly intestinal-type, arises via the carcinogenic gastric cascade initiated by *Helicobacter (H.) pylori* and involves several histological changes: Active inflammation, non-atrophic chronic gastritis, multifocal atrophic gastritis (AG), intestinal metaplasia (IM), dysplasia, and cancer (4-6). A previous study demonstrated an increased risk of IM in patients with high levels of bile acid reflux, hence DGR might contribute to the development of precancerous lesions and GC (7).

The results of studies investigating the relationship between *H. pylori* and bile reflux are contradictory. Some studies report that *H. pylori* colonization decreases as a result of high levels of bile acids (8), while others revealed a positive relationship between *H. pylori* and DGR, where DGR was found to increase the presence of bacteria (9,10).

The present study questioned the importance of concomitant active *H. pylori* infection on endoscopic and histologic gastric changes in patients with biliary aggression. The objective was to investigate their association with possible demographic and clinical predictors (digestive symptoms, drug consumption, social habits, comorbidities) in order to identify potential strategies to limit progression toward stomach cancer, in a population with a high prevalence of long-standing *H. pylori* infection.

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## Patients and methods

*Subjects and data collection*. A total of 2,014 patients with dyspeptic complaints or anemia, hospitalized at the Medical Clinic No. 2 in Târgu Mureș Emergency County Hospital, Romania, underwent an upper digestive endoscopy (UDE) between January 2016 and December 2019. The present study involved those patients diagnosed with DGR following UDE (n=560) divided into two groups: Study group (Bile reflux + *H. pylori*), 195 patients with *H. pylori* infection confirmed upon biopsy specimens utilizing usual histochemical stains or immunohistochemical methods; and Control group (bile reflux), 365 patients negative for *H. pylori* infection on all biopsy sites or immunohistochemistry when performed.

Bile reflux diagnosis was based on detection of DGR and a large bile pool in the stomach during the endoscopic examination, in patients with a fasting period of >10 h.

Five random biopsy specimens were taken and examined for each patient: Two from the antrum, two from the corpus (the greater and the lesser curvature), and one from incisura angularis. The tissue samples were fixed in formalin, embedded in paraffin, and stained with routine hematoxylin and eosin, PAS-Alcian blue and modified Giemsa, followed by microscopic evaluation. *H. pylori* infection was considered negative if the bacteria were absent from all biopsy sites and positive if identified in at least one biopsy sample.

When no organism was detected using histochemical stains, but a high suspicion of infection existed due to persistent inflammation, particularly for patients with proton-pump inhibitor (PPI) therapy, the pathologist performed immunohistochemistry staining. The immunohistochemical staining was performed with FLEX polyclonal rabbit anti-*Helicobacter pylori* antibody, Ready to Use (Dako Autostainer) and using EnVision FLEX, High pH as a visualization system (Fig. 1).

In the database were noted the clinical examination, drug exposure, comorbidities, symptoms, and social habits of the patients. Exclusion criteria were acute bleeding episodes, previous gastrectomy or *H. pylori* eradication therapy, dysplasia, gastric/esophageal neoplasms, and missing data (drug exposure, social habits, biopsies).

Recorded digestive symptoms included epigastric pain, heartburn, flatulence, and nausea/vomiting. We considered chronic proton pump inhibitor therapy (pantoprazole, esomeprazole, omeprazole) if administered at regular doses for ≥3 months before investigation.

Anemia and diabetes mellitus were registered when the diagnosis was made based on international clinical guideline criteria. For anemia, the cut-off values of hemoglobin levels according to the WHO definitions were: 12 g/dl (7.5 mmol/l) in women and <13 g/dl (<8.1 mmol/l) in men (11). We considered alcohol consumption if patients declared a consumption of ≥10 units (10 ml) of pure alcohol weekly. Patients registered as smokers were those that smoked >5 cigarettes/day, including quitters during the past 5 years.

*Additional endoscopic and histologic aspects*. During the endoscopic examination of the stomach the following data were recorded: Mucosal fragility, hyperemia, edema, petechiae/erosions/ulcers, hiatal hernia, and bile reflux. In order to describe the endoscopic appearance of reflux esophagitis and grade its severity, Los Angeles classification system was utilized (12). In the present study, esophagitis was recorded as present or absent.

We assessed gastro-duodenal lesions using a modified Lanza score: Grade 0, no mucosal lesions; Grade 1, one erosion (mucosal defect <5 mm) or petechiae (hemorrhagic area without mucosal defect); Grade 2, 2-10 erosions/petechiae; Grade 3, >10 erosions/petechiae; Grade 4, gastric ulcer (defect >5 mm in diameter). Patients having Lanza scores between 0 and 2 were registered with no/mild endoscopic lesions, and those with scores of 3 and 4 were diagnosed with severe endoscopic lesions (13).

Patients with foveolar hyperplasia, vascular dilation of superficial mucosal capillaries, fibromuscular replacement of the lamina propria and without inflammation were diagnosed with reactive gastropathy.

Atrophy was registered as the loss of normal glands in both the antrum/corpus. IM has various aspects on histology. The pathologist considered complete IM when some parts of the gastric epithelium had a small intestinal epithelium aspect, with eosinophilic enterocytes, goblet cells and brush border. Incomplete IM was defined as colonic epithelium in gastric mucosa, containing mucin droplets with irregular form and different sizes, as well as the absence of the brush border. The Updated Sydney System was used to assess the intensity of mononuclear inflammatory cellular infiltrates and inflammatory activity (neutrophilic infiltrations) in the diagnosis of gastritis. The degree of the atrophy and IM was defined using the OLGA/OLGIM (Operative Link on Gastritis/IM Assessment) (14). In addition, patients with antral-type glands in the body of the stomach were diagnosed with pseudopyloric metaplasia.

*Statistical analysis*. For statistical analysis, JASP (JASP graphical program for statistical analysis supported by the University of Amsterdam) program v.0.12.1 (https://jasp-stats.org/) was applied. Mean ± standard deviation or absolute (relative) frequencies were used to summarize the demographic and clinical data. To identify significant differences in terms of quantitative demographic characteristics, Mann Whitney U test was applied, while for comparison of categorical variables the Chi-square or Fisher's exact test were utilized, both followed by univariate logistic regression analysis. All significant factors and factors whose unadjusted estimated significance level was P<0.25 in univariate analysis were selected as candidates for multivariate logistic regression. The partial likelihood ratio test was applied to decide the analyzed variables in the final model. The odds ratio (OR) and 95% CI quantified the magnitude of the association. A two-sided P-value <0.05 was considered statistically significant in all tests.

## Results

The observed relative frequency of *H. pylori* infection among patients with bile reflux was 34.82%. There was no significant difference in terms of age between the Study and Control groups: Median (Q1-Q3) 62 years (52-72) for controls vs. 60 years (50.5-69.5) for cases, U=38206, P=0.151,

Table I. Group differences regarding the studied factors in patients with DGR ± *H. pylori* infection.

| Variables | Bile reflux + *H. pylori* (study group) (n$_1$=195) | | Bile reflux (control group) (n$_2$=365) | | P-value[a] |
| --- | --- | --- | --- | --- | --- |
| | No. | % | No. | % | |
| Age >65 years | 65 | 33.3 | 150 | 41 | 0.0800 |
| Female sex | 98 | 50.3 | 199 | 54.6 | 0.3700 |
| Premalignant gastric lesions | 73 | 37.4 | 118 | 32.3 | 0.2200 |
| Severe endoscopic lesions | 64 | 45.1 | 104 | 28.4 | 0.2800 |
| Anemia | 32 | 16.4 | 67 | 18.3 | 0.6400 |
| Esophagitis | 43 | 22.0 | 72 | 19.7 | 0.5100 |
| Diabetes | 34 | 17.4 | 62 | 16.9 | 0.9000 |
| Heartburn | 58 | 29.7 | 98 | 26.8 | 0.4800 |
| Epigastric pain | 103 | 52.8 | 156 | 42.7 | **0.0261** |
| Nausea/Vomiting | 33 | 16.9 | 55 | 15.0 | 0.6200 |
| Flatulence | 50 | 25.6 | 67 | 18.3 | **0.0496** |
| Smoking[b] | 31 | 15.8 | 34 | 9.3 | **0.0263** |
| Alcohol consumption[c] | 40 | 20.5 | 54 | 14.7 | 0.0900 |
| PPI therapy | 73 | 37.4 | 185 | 50.6 | **0.0033** |

[a]Obtained from Chi-square or Fisher's exact tests; [b]Over 5 cigarettes/day; [c]More than 2 units/day, 1 unit=10 ml pure alcohol. *H. pylori*, *Helicobacter pylori*; DGR, duodenogastric reflux; PPI, proton-pump inhibitor; OR, odds ratio; CI, confidence interval. P-values indicative of a significant result are presented in bold print.
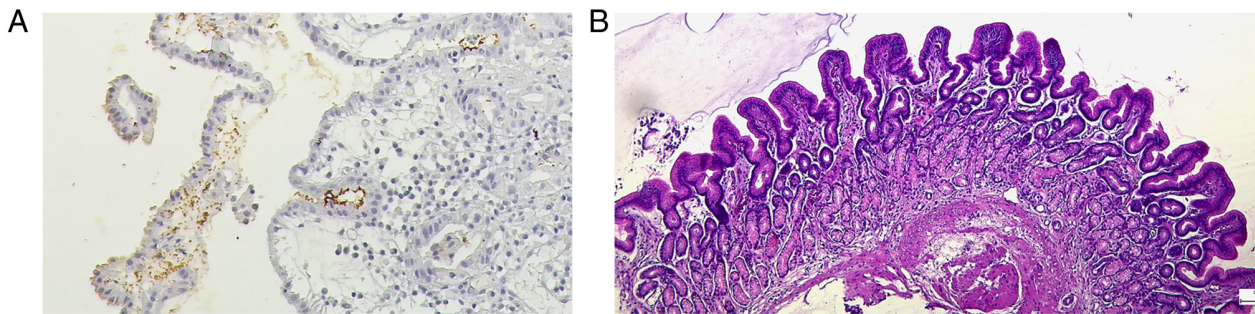


Figure 1. Histologic aspects in *Helicobacter pylori* gastritis compared to reactive gastropathy. (A) Immunohistochemical staining for *Helicobacter pylori*: With numerous positive microorganisms attached to the luminal surface of the foveolar cells; magnification, x10. (B) Reactive gastropathy: Foveolar hyperplasia with mild changes in the lamina propria, but without any inflammation. Hematoxylin and eosin staining; magnification, x4.

but patients >65 years, although without a statistically significant level, were more frequent in the Bile reflux group (without *H. pylori* infection). The differences between demographic and clinical characteristics are presented in Table I. Severe endoscopic lesions (score 3 and 4) and premalignant gastric lesions (IM and AG) were more common in patients with DGR reflux and *H. pylori* infection [45.1% (64) vs. 28.4% (104) and 37.4% (73) vs. 32.3% (118), respectively], but without a statistically significant association (Table I).

In the Bile reflux group, the stomach histology most frequently demonstrated reactive gastropathy (44.4% patients), followed by inactive chronic gastritis (40.5%). Also, 15.1% of the patients were without histologic lesions.

The distribution of premalignant gastric lesions was similar between the Study and Control groups. Patients presented more frequent both IM and AG, but there were 41% cases and 39% controls of IM alone. AG, without IM, was found only in a small percentage of patients with premalignant gastric lesions (Table II).

In the univariate logistic regression model, smoking was a significant factor associated with DGR and *H. pylori* infection, while pre-treatment with PPI had a negative effect. Regarding the symptomatology, epigastric pain and flatulence were significant factors associated with *H. pylori* infection and bile reflux (Table III).

According to the multivariate logistic analysis results, in patients with DGR, smoking was significantly associated with *H. pylori* infection, while PPI therapy remained negatively associated with the immunohistochemically confirmed infection. The odds of *H. pylori* infection increased by 1.88

Table II. Premalignant gastric lesion distribution.

| Premalignant gastric lesions (n=191) | Bile reflux + *H. pylori* (Study group) ($n_1$=73) | | Bile reflux (Control group) ($n_2$=118) | | P-value[a] | OR | 95% CI |
|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | | | |
| IM | 30 | 41.1 | 46 | 39 | 0.80 | 1.07 | 0.65 to 1.75 |
| AG | 2 | 2.7 | 8 | 6.8 | 0.32 | 0.38 | 0.07 to 1.87 |
| IM + AG | 41 | 56.2 | 64 | 54.2 | 0.88 | 1.08 | 0.60 to 1.94 |

[a]Obtained from Chi-square test or Fisher's exact test. *H. pylori*, *Helicobacter pylori*; IM, intestinal metaplasia; AG, atrophic gastritis; OR, odds ratio; CI, confidence interval.

Table III. Results from the univariate binary logistic regression.

| Variable | Statistics Z | P-value[a] | Crude OR | 95% CI |
|---|---|---|---|---|
| Age >65 years | -1.79 | 0.07 | 0.71 | 0.49 to 1.03 |
| Female sex | -0.96 | 0.33 | 0.84 | 0.59 to 1.19 |
| Severe endoscopic lesions | 1.06 | 0.28 | 1.22 | 0.84 to 1.78 |
| Anemia | -0.57 | 0.56 | 0.87 | 0.54 to 1.38 |
| Esophagitis | 0.64 | 0.51 | 1.15 | 0.75 to 1.76 |
| Diabetes | 0.13 | 0.89 | 1.03 | 0.65 to 1.63 |
| Heartburn | 0.72 | 0.46 | 1.15 | 0.78 to 1.69 |
| Epigastric pain | 2.27 | **0.023** | 1.50 | 1.05 to 2.12 |
| Nausea/Vomiting | 0.57 | 0.56 | 1.14 | 0.71 to 1.84 |
| Flatulence | 2.09 | **0.036** | 1.56 | 1.02 to 2.37 |
| Smoking[b] | 2.29 | **0.022** | 1.84 | 1.09 to 3.10 |
| Alcohol consumption[c] | 1.17 | 0.08 | 1.48 | 0.94 to 2.33 |
| PPI therapy | -2.98 | **0.003** | 0.58 | 0.40 to 0.83 |

[a]Crude P-values obtained from Wald's test; Response variable: Presence of *Helicobacter* (*H.*) *pylori* infection in patients with biliary reflux. [b]Over 5 cigarettes/day; [c]More than 2 units/day, 1 unit: 10 ml pure alcohol. Crude OR, unadjusted odds ratio; CI, confidence interval; PPI therapy, proton-pump inhibitors. P-values indicative of a significant result are presented in bold print.

Table IV. Final multivariable logistic regression model.

| Variables | b[a] | SE | P-value[b] | Adjusted OR | 95% CI |
|---|---|---|---|---|---|
| Smoking | 0.63 | 0.27 | **0.020** | 1.88 | 1.10 to 3.21 |
| PPI therapy | -0.67 | 0.19 | **<0.001** | 0.50 | 0.35 to 0.73 |
| Flatulence | 0.36 | 0.21 | 0.090 | 1.44 | 0.93 to 2.21 |
| Epigastric pain | 0.52 | 0.18 | **0.005** | 1.69 | 1.17 to 2.45 |
| Intercept | -0.73 | 0.14 | **<0.001** | 0.47 | 0.35 to 0.64 |

[a]Estimated unstandardized regression coefficients. [b]Wald's test adjusted P-value. SE, standard error; CI, confidence interval; PPI therapy, proton-pump inhibitors. P-values indicative of a significant result are presented in bold print.

(95% CI 1.10 to 3.21) in smokers with bile reflux, effect adjusted for other covariates. Epigastric pain was related to *H. pylori*-positive patients, while flatulence had only a tendency towards statistical significance (P=0.09) (Table IV).

The final multivariable logistic regression model was statistically significant, $\chi^2$(555)=26.52, P<0.001. The model correctly classified 66% of patients and the Brier score of 0.21 indicates a good accuracy of the model.

## Discussion

The diagnosis and clinical consequences of DGR are challenging, particularly for patients without known risk factors (gastric surgery) (15). High amounts of bile acids can cause direct gastric mucosal injury by its components, triggering inflammation (16). Because bile reflux and *H. pylori* often coexist and both are involved in the pathogenesis of gastritis, research has examined the relationship between these two entities. All patients included in this study had endoscopic criteria for biliary reflux and potential chemical aggression of the gastric mucosa. Only less than half of patients without active *H. pylori* infection (Control group) presented histologic features of gastropathy (44.4%), while 15% had no histologic changes. The absence of histologic gastropathy supports less aggressive or recurrent exposure to bile acid or no biopsy from those areas with chemical aggression. In patients with active *H. pylori* infection, the chemical aggression is 'covered' by histologic inflammatory changes. Reexamination after eradication therapy may appreciate the role of each factor in the changes in gastric mucosa.

A recent article suggests a molecular mechanism related to the FXR/NF-κB signaling pathway, CDX2, and MUC2 implicated in IM occurrence and progression in patients with bile reflux (17). In addition, researchers have questioned the role of cytokines and their gene variants in premalignant gastric lesion development in patients with *H. pylori* infection (18). The risk of GC, mucosal aggression, symptoms and interaction with *H. pylori* infection are important issues in managing patients with DGR in daily practice as *H. pylori* favors the release of gastrin in the antrum, decreases peristaltic movements, and determines bile reflux (16). Although a higher prevalence of IM has been reported in patients with both DGR and *H. pylori* (10), in the studied population, *H. pylori* infection over bile reflux appeared not to exert significant additional influence on IM and AG development.

Severe endoscopic lesions were not significantly associated with concomitant *H. pylori* infection in the studied groups, sustaining previous findings in patients with gastric surgery in whom *H. pylori* did not play a substantial role in ulcer recurrence (19).

In the present study, epigastric pain was the most common complaint in both groups and was significantly associated with *H. pylori* infection, an effect that remained significant also in multivariate analysis. Another study investigating the symptoms in patients with *H. pylori* infection and bile reflux, reported nausea as the main complaint in patients with DGR, while in patients with *H. pylori* gastritis, epigastric pain was the most frequent symptom, followed by heartburn (20). Nevertheless, *H. pylori* infection was detected in only 40% of dyspeptic patients in the Romanian population, the role of other underlying conditions playing an important role (21).

The role of bile reflux in reflux esophagitis pathogenesis assumes chemical injury, but a recent study suggests that acidic bile salts induce esophagitis by a cytokine-mediated inflammation injury, driven by hypoxia-inducible factor 2α (HIF-2α) (22). Regarding the relationship between *H. pylori* and esophagitis, a meta-analysis showed that eradication of *H. pylori* increased the risk of esophagitis development, compared with patients without eradication. However, in the present study, the number of cases with esophagitis was similar between the two groups (23).

In the studied population, patients with DGR and negative for *H. pylori* infection were found to be consuming more frequent proton-pump inhibitors (PPIs). The effect of PPIs on decreasing the bacterial load in biopsy samples is well-known (24); therefore, to counteract the possible false-negative results when the suspicion of infection persisted due to inflammatory changes on histology, pathologists performed immunohistochemistry staining. High number of PPI prescriptions may be explained by the rich symptomatology attributed to both gastric acid secretion and DGR.

In the studied population, the multivariate logistic analysis revealed smoking is associated with *H. pylori* infection in patients with DGR. This result is in line with studies demonstrating that smoking affects gastro-duodenal motility and favors bile reflux into the stomach. Furthermore, smoking imbalances the antioxidant equilibrium and increases the risk of *H. pylori* infection (25).

The strength of the present study is the systematic histologic assessment of both antrum and corpus changes in patients with biliary reflux on endoscopy, tied with a thorough description of the most relevant clinical and endoscopic findings. Patients with a non-invasive diagnosis of *H. pylori* infection often complain about the persistence of symptoms after eradication therapy. Identifying associations between bile reflux and clinicopathological parameters may offer possible strategies to manage the symptoms and follow-up of this group of patients. The study has all the limitations of a retrospective observational study. The lack of bile reflux measurement and pH monitoring, and patients enrolled from the same geographical area are aspects to be improved.

In conclusion, in the studied population, *H. pylori* infection over DGR did not exert significant additional influence on the severity of endoscopic lesions, neither in premalignant gastric lesion development. Epigastric pain is more frequent in patients with bile reflux and *H. pylori* infection than in uninfected patients. Smoking was associated with *H. pylori* infection in patients with DGR, while *H. pylori* infection did not influence esophagitis occurrence. In patients with DGR diagnosis, PPI therapy had a negative association with immunohistochemically assessed *H. pylori* infection active gastritis.

## Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on request.

**Authors' contributions**

Conceptualization of the study design was achieved by AN and AS. Methodology was the responsibility of AN. Formal analysis was conducted by AN and AS. Study investigation was conducted by AN and SM. Resources were obtained by AN and data curation was performed by AN and AS. Writing and original draft preparation was done by AS, AN and SM. Writing, review and editing was done by AN and AS. Supervision was conducted by AN, and project administration and funding acquisition were the responsibility of AN. All authors have read and agreed to the published version of the manuscript.

**Ethics approval and consent to participate**

The research was approved by the Ethics Committee of the University of Medicine and Pharmacy of Târgu Mureș, Romania (282, 19.07.2019). The patient informed consent was obtained for regular endoscopic investigation.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors state that they have no competing interests.

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