

Spontaneous clearance of *Helicobacter pylori* after pylorus-preserving gastrectomy for gastric cancer

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Abstract. Residual mucosa in the gastric stump after pylorus-preserving gastrectomy (PPG) is considered a risk factor for the development of gastric stump carcinoma (GSC). Duodenogastric reflux (DGR) and *Helicobacter pylori* (*H. pylori*) infection are suspected to contribute to the development of GSC. The aim of this study was to investigate the prevalence of *H. pylori* in the residual stomach after PPG for gastric cancer and to assess factors associated with the presence of *H. pylori*. We investigated 72 patients who had undergone PPG at least 1 year prior to the study and were confirmed to be positive for *H. pylori* infection on presurgical endoscopic biopsy. The extent of DGR, the prevalence of *H. pylori* infection based on *H. pylori* stool antigen (HpSA) tests and the severity of gastritis were analyzed in these post-PPG patients. None of the patients had DGR, as shown by ^{99m}Tc-PMT. Of the 72 post-PPG patients, 33 (46%) were positive for HpSA. The prevalence of *H. pylori* infection was significantly lower after surgery than before surgery. The endoscopic severity of remnant gastritis, as well as histological inflammation and activity, were higher in *H. pylori*-positive patients than in *H. pylori*-negative patients. In conclusion, some patients who undergo PPG and are negative for DGR experience spontaneous clearance of *H. pylori* infection.

Introduction

Gastrectomy is a risk factor for the long-term development of gastric stump carcinoma (GSC) (1). The formation of a gastric

stump after surgery is considered a precancerous condition (2). Many factors appear to be involved in the etiopathogenesis of GSC, including achlorhydria, hypergastrinemia and biliary reflux, Epstein-Barr virus and *Helicobacter pylori* (*H. pylori*) infection, atrophic gastritis, and polymorphisms in the genes encoding interleukin-1 β and cyclooxygenase-2 (3-5).

Patients may develop GSC following distal gastrectomy for benign disease. For example, a large population-based study showed that patients who underwent gastric resection for benign disease had an increased risk of cancer in the gastric remnant ≥ 30 years later (6). In contrast, a small case series showed that patients may develop GSC following distal gastrectomy for cancer (7,8). Early detection of GSC is important, and strict surveillance for a minimum of 10 years has been recommended after initial gastrectomy for gastric cancer (9,10). The incidence of GSC may be higher after the Billroth II procedure than after the Billroth I procedure (7,11), because higher amounts of duodenal contents containing bile persist in the gastric stump after undergoing Billroth II gastrectomy than after Billroth I gastrectomy (12). These findings therefore suggest that duodenogastric reflux (DGR) may be associated with the development of GSC.

H. pylori infection is thought to be a significant risk factor for gastric cancer (13,14), with recent epidemiologic evidence suggesting the involvement of *H. pylori* in the carcinogenic process (15). In addition, *H. pylori* eradication has been associated with a reduced likelihood of metachronous cancer development and with tumor growth inhibition, since eradication is associated with the healing of background gastric mucosa (16).

GSC may also be related to *H. pylori* infection. Patients who undergo distal gastric resection have an increased risk of developing GSC, primarily because of DGR. DGR correlates with spontaneous eradication of *H. pylori* infection (17,18), and facilitates the survival of *H. pylori* in the gastric stump, after distal gastrectomy (19).

Proton pump inhibitor (PPI)-based standard therapy is just as effective for eradicating *H. pylori* from the remnant stomach as from the non-surgically treated stomach (20). Eradication therapy results in significant improvements in inflammation and atrophy of the mucosal layer in the remnant stomach after

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Abbreviations: *H. pylori*, *Helicobacter pylori*; GSC, gastric stump carcinoma; PPG, pylorus-preserving gastrectomy; PPI, Proton pump inhibitor; DGR, duodenogastric reflux

Key words: *Helicobacter pylori* eradication, pylorus-preserving gastrectomy, gastric stump carcinoma, duodenogastric reflux

early gastric cancer surgery (21). These findings support the role of *H. pylori* in gastric carcinogenesis and suggest that *H. pylori* eradication therapy may prevent the development of metachronous gastric cancer after gastric resection (22).

Pylorus-preserving gastrectomy (PPG), including transectional resection (TR) and local resection (LR), in patients with early gastric cancer along with lymphatic basin dissection, has been found to modulate gastric emptying and prevent DGR (23,24). Moreover, sleeve gastrectomy has been reported to lead to *H. pylori* eradication (25). We investigated the prevalence of *H. pylori* in the residual stomach after PPG for gastric cancer, as well as the correlations between *H. pylori* positivity and the clinical characteristics and severity of gastritis in the residual stomach.

Materials and methods

Patients. Patients who underwent PPG, including transectional resection (TR) and local resection (LR), at least 1 year prior to this study and were followed up as outpatients in our department were selected for this study. All patients were positive for *H. pylori* infection on endoscopic biopsy before surgery. Subjects taking H₂ receptor antagonists, proton pump inhibitors (PPIs), non-steroidal anti-inflammatory drugs, antibiotics, or bismuth salts, and those who had undergone *H. pylori*-eradication therapy were excluded. Seventy-two patients agreed to participate in the trial, with all providing prior, written, informed consent. The median patient age was 62 years (range 31-85 years). Forty-eight subjects were male and 24 were female. All patients had undergone PPG for early gastric cancer. The median time from surgery was 5 years (range 1-10 years). None of these patients had experienced local recurrence or metastasis of their original tumor. Two patients developed GSC 9 years after PPG. Of the 72 patients, 46 had undergone TR (23) and 26 had undergone LR; these 2 groups did not differ in regards to patient characteristics, including mean age, gender, distribution, or mean time following surgery.

DGR. The degree of DGR was assessed by using hepatobiliary scintigraphy, by monitoring pH, and by endoscopic examination.

Hepatobiliary scintigraphy. Eighteen patients were selected. After an overnight fast, each patient received an intravenous injection of 37 MBq of ^{99m}Tc N-pyridoxyl-5-methyltryptophan (^{99m}Tc-PMT; Japan Medi-Physics, Japan). Patients then underwent serial hepatobiliary scanning in the sitting position using a gamma camera, with images taken at 0, 10, 20, 30, 45, 60, 90 and 120 min. A region of interest (ROI) corresponding to the remnant stomach was outlined in anterior views, and the radioactivity in each ROI was measured and expressed as a percentage of the radioactivity at time 0 (i.e., reflux amount).

Measurement of pH in the remnant stomach. Before assessment, the pH monitor was calibrated, i.e., the tip of the probe was introduced into a liquid and stabilized. The calibrated probe was placed transnasally in the remnant stomach and taped in place, with measurements started after assuring that the equipment was correctly placed. The pH was measured

for 24 h, following which, all data from the device were transferred to a personal computer and analyzed.

Endoscopic examination. All patients underwent endoscopic examination every 6 months following surgery. During upper gastrointestinal endoscopy, performed after an overnight fast, tissue specimens were sampled from the greater curvature of the antrum and the fornix.

Detection of *H. pylori*. Stool samples of all patients were collected and analyzed for *H. pylori* antigen using enzyme immunoassays (HpSA, Premier Platinum HpSA; Meridian Diagnostics Inc., Cincinnati, OH, USA) in accordance with the manufacturer's instructions. Briefly, diluted fecal samples and a peroxidase-conjugated polyclonal antibody were added to the microwells containing polyclonal antibodies to *H. pylori* and incubated for 1 h at room temperature. The wells were washed to remove unbound materials. Substrate was added and the wells were incubated for 10 min at room temperature. The presence of bound *H. pylori* antigens was demonstrated by a change in color from blue to yellow. A stop solution was added, and spectrophotometric analysis was performed at 450 nm. Absorbances <0.140 were considered negative; those from 0.140-0.159 were considered equivocal; and those >0.160 were considered positive. In these assays, buffer mixed with inactivated *H. pylori* antigen was used as a positive control, and buffer mixed with preservative was used as a negative control.

Histological assessment of biopsy specimens. Biopsy specimens from each site of the stomach were oriented on filter paper and immediately fixed in 10% buffered formalin. Paraffin-processed sections were cut at 3 levels and stained with hematoxylin and eosin (H&E). The sections were examined in a blinded manner by a single pathologist and specifically assessed for severity of gastritis using the updated Sydney system. The degree of inflammation, activity, atrophy and intestinal metaplasia at each site was graded as normal, mild, moderate or severe.

Statistical analysis. Differences between groups were analyzed using the Student's t-test, the Mann-Whitney rank sum test, Fisher's exact test, or the log-rank test, as appropriate. All statistical analyses were performed using StatView software (SAS Co., Berkeley, CA, USA), with p-value <0.05 considered to indicate a statistically significant result.

Ethics. The study was performed according to the Declaration of Helsinki and approved by the Regional Ethics Committee of Kanazawa University.

Results

Environment of the remnant stomach after PPG. Of the 18 patients tested, none showed detectable ^{99m}Tc-PMT in the remnant stomach, indicating an absence of bile reflux in patients who had undergone PPG at least 1 year earlier. Representative findings of ^{99m}Tc-PMT and pH monitoring in a post-LR patient showed no DGR and a mean intragastric pH of 5.2 over 24 h (Fig. 1).

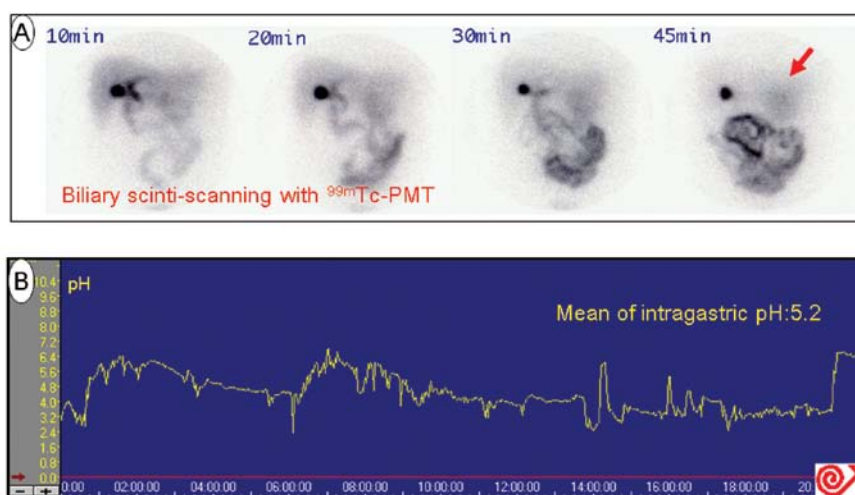


Figure 1. Scintigraphy and pH monitoring in a representative patient. (A) ^{99m}Tc -PMT showing a complete absence of duodenogastric reflux (DGR). Red arrow indicates remnant stomach. (B) Measurement of intra-gastric pH over 24 h, with a mean intra-gastric pH of 5.2.

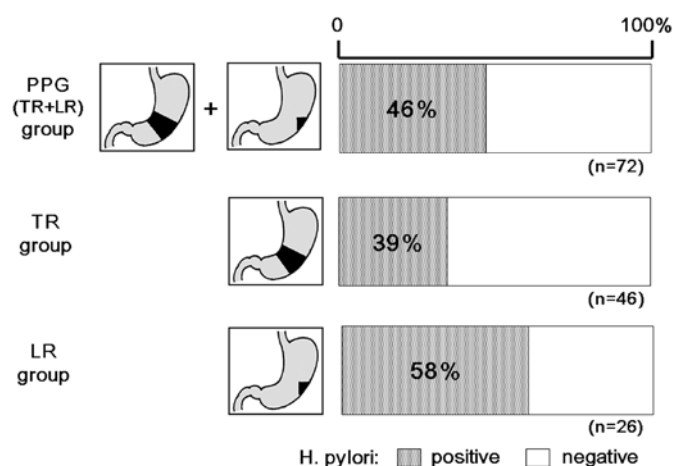


Figure 2. Incidence of *H. pylori* infection before and after pylorus-preserving gastrectomy (PPG). All patients were confirmed as being *H. pylori* infection-positive on endoscopic biopsy before surgery. The prevalence of *H. pylori* infection was significantly reduced after PPG, whether transectional resection (TR) or local resection (LR).

Upper gastrointestinal endoscopy showed that 2 of the 72 (3%) patients had mild DGR.

Presence of *H. pylori*. Of the 72 patients, 33 (46%) were positive for HpSA, including 18 of the 46 (39%) patients who had undergone TR and 16 of the 26 (58%) patients who had undergone LR. The overall prevalence of *H. pylori* infection was significantly lower after PPG than before PPG (Fig. 2).

Degree of gastritis. We assessed the relationship between *H. pylori* infection and the endoscopic severity of remnant gastritis. Endoscopically, we observed redness and edema throughout the remnant gastric mucosa, with the incidence of both being higher in *H. pylori*-positive than in *H. pylori*-negative patients. Moreover, histological evaluation of the biopsy specimens taken from the greater curvature of the antrum and the fornix showed that both inflammation and activity were

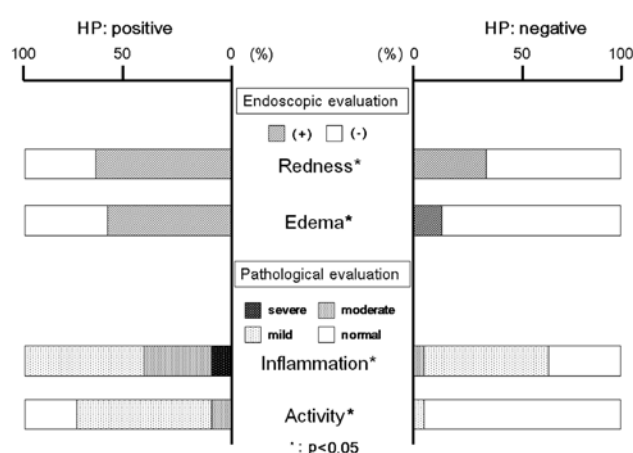


Figure 3. Associations between endoscopic and histological findings with *H. pylori* infection in the remnant stomach. The rates of redness, edema, inflammation and activity were significantly higher in the post-pylorus-preserving gastrectomy (PPG) mucosae of *H. pylori*-positive patients than in the post-PPG mucosae of *H. pylori*-negative patients.

higher in *H. pylori*-positive than in the *H. pylori*-negative patients (Fig. 3).

Outcomes in post-PPG patients with and without *H. pylori* infection. We found that 2 patients had GSC with persistent *H. pylori* infection 9 years after PPG (Fig. 4A and B), but both were negative for bile reflux using ^{99m}Tc -PMT.

Of the 33 patients positive for *H. pylori* after PPG, 6 underwent *H. pylori* eradication using standardized methods, with eradication in all 6 being successful. One of these 6 patients had an ulcer, and another had reflux esophagitis after eradication (Fig. 4C and D), but both were successfully treated with PPIs.

Discussion

To the best of our knowledge, this is the first study to compare the spontaneous reduction in *H. pylori* infection and the

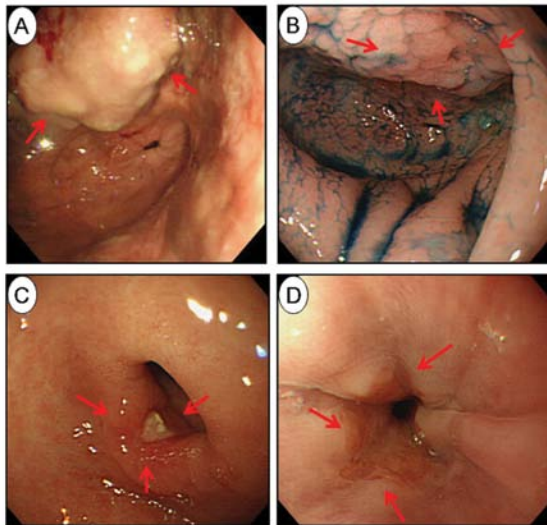


Figure 4. Complications in patients after pylorus-preserving gastrectomy (PPG). (A and B) Two patients with residual gastric cancer and persistent *H. pylori* infection 9 years after PPG. (C) One patient had an ulcer after eradication. (D) One patient had GERD after eradication.

presence of mucosal lesions in the remnant stomach after PPG for gastric cancer.

The presence of residual mucosa in the gastric stump is considered a risk factor for GSC, as well as a precancerous condition, independent of indications for surgery (22). Although there are more sensitive methods for detecting *H. pylori*, we assessed *H. pylori* infection status using stool HpSA tests. The use of stool tests may explain the lower prevalence of *H. pylori* infection among our patients than in a previous study (26). However, bacterial culture and histopathological examinations are direct but more invasive methods, whereas the urea breath and stool HpSA tests are both indirect and non-invasive methods of detecting *H. pylori* (27). Comparisons of urease and stool HpSA tests with histopathology showed that the sensitivity and specificity of the urease tests were 62.2 and 100% respectively, whereas the sensitivity and specificity of the stool tests were 68.9 and 100% respectively, indicating that the results obtained with biopsy urease and HpSA tests were generally similar (28), and that stool HpSA tests are generally useful for the diagnosis of *H. pylori* infection.

Because it constitutes a precancerous condition, the resected stomach offers an opportunity to assess the factors involved in gastric carcinogenesis. Bile reflux interferes with the prevalence of *H. pylori* infection (17,18). Roux-en-Y reconstruction following distal gastrectomy has been shown to be superior to Billroth I and II reconstruction in preventing remnant gastritis, as the former reduces DGR (29). Therefore, Roux-en-Y reconstruction may reduce *H. pylori* infection by preventing bile reflux and gastritis (30). The prevalence of *H. pylori* infection was found to be low following jejunal pouch interposition and Roux-en-Y reconstruction, which prevent bile reflux, but high in patients who underwent Billroth I and II reconstruction. DGR was rare in patients who underwent jejunal pouch interposition and Roux-en-Y reconstruction, but was often observed in patients who underwent Billroth I and II reconstruction, suggesting a positive relationship between DGR

and *H. pylori* infection (19). We found that the PPG procedure prevented bile reflux, as shown by ^{99m}Tc -PMT, and reduced the prevalence of *H. pylori* significantly when compared with its prevalence prior to PPG, providing further evidence of the positive relationship between DGR and *H. pylori* infection.

Routine post-surgical treatment with the antibiotic cephalosporin may affect *H. pylori* infection. However, an evaluation of 8 *H. pylori*-positive colorectal cancer patients showed that none became *H. pylori*-negative after surgery and antibiotic treatment. These findings suggest that post-operative antibiotic treatment is not associated with eradication of *H. pylori* infection after surgery.

We found that 2 patients had residual gastric cancer with persistent *H. pylori* infection 9 years after PPG. Of the 33 *H. pylori*-positive patients after PPG, 6 underwent *H. pylori* eradication using standardized methods, with eradication in all 6 being successful. One of these patients had GERD, and another had an ulcer after eradication, but both were successfully treated with PPIs, suggesting that eradication therapy may prevent GCS. PPI-based therapy was as effective in eradication *H. pylori* in remnant stomachs as in unoperated stomachs, with eradication therapy significantly decreasing inflammatory cell infiltration of the mucosal layer (20,31). In addition, eradication therapy decreased the Ki-67 labeling index and almost normalized tissue IL-8 levels, suggesting that *H. pylori* eradication may reduce the risk of *H. pylori*-associated carcinogenesis in patients who have undergone gastrectomy for early gastric cancer (32).

Our findings suggest that PPG itself may lead to *H. pylori* eradication. Further clinical studies on larger populations are needed to address this issue and to formulate appropriate guidelines for this relatively new procedure.

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