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Quantitative Determinations of Duodenogastric Reflux, Prevalence of *Helicobacter pylori* Infection, and Concentrations of Interleukin-8

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Abstract. Billroth I or II reconstruction after distal gastrectomy often is associated with inflammation in the gastric remnant. We sought to determine which reconstructive procedure was most effective in preventing such remnant gastritis. Patients undergoing curative distal gastrectomy for cancer ($n = 82$) were classified as group A (Roux-en-Y, $n = 22$); group B (Billroth I, $n = 40$); or group C (Billroth II, $n = 20$). Interleukin (IL)-8 concentrations in gastric mucosa were measured 3 months after surgery. In the absence of *Helicobacter pylori* infection, IL-8 concentrations were 13, 56, and 87 pg/mg protein in groups A, B, and C, respectively ($p < 0.05$). In the presence of *H. pylori* infection, IL-8 concentrations were 61, 161, and 234 pg/mg protein in groups A, B, and C ($p < 0.01$). Roux-en-Y reconstruction is better able to prevent remnant gastritis than either the Billroth I or II procedure as judged from IL-8 concentrations in gastric remnant mucosa.

Following subtotal gastrectomy, Billroth I (gastroduodenostomy) or Billroth II (gastrojejunostomy) reconstructions commonly are performed [1]. However, these reconstructive procedures sometimes are associated with inflammation in the gastric remnant [2]. Remnant gastritis causes such symptoms as dyspepsia, epigastralgia, and heartburn, which adversely affect quality of life for patients [3]. Mechanisms underlying development of remnant gastritis after distal gastrectomy remain poorly understood, but duodenogastric reflux is thought to be a major causative factor [4].

Inflammation in the gastric remnant ordinarily is evaluated endoscopically [5], but such assessment is subjective. Gastritis also has been graded pathologically in terms of infiltrating neutrophils using the updated Sydney system [6]. Significantly, cytokines, important mediators in inflammatory responses, can be assayed. Interleukin (IL)-8, a member of the chemokine family of chemoattractant cytokines, is a sensitive marker of inflammation. Many investigators have found elevated gastric mucosal IL-8 concentrations in *Helicobacter pylori* infection [7, 8], but no report has compared IL-8 concentrations in the gastric remnant after various reconstructive procedures following distal gastrectomy. We have observed that reconstruction with biliary diversion (Roux-en-Y) decreased the likelihood of remnant gastritis [9]. The present report considers effects of various reconstructions on remnant gastritis as indicated by tissue concentrations of IL-8. The influence of *H. pylori* infection also is assessed.

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Materials and Methods

Patients

Among patients with gastric cancer who underwent curative distal gastrectomy in our department between May 1999 and July 2001, 82 subjects (52 men, 30 women; mean age, 62.3 years; range, 38 to 83 years) were studied. None had been taking any nonsteroidal antiinflammatory drug, proton-pump inhibitor, or prokinetic drug during the preceding 2 months. In all patients the operation was performed or supervised by a single surgeon (H. Osugi). Following distal gastrectomy with lymph node dissection, Billroth I (end-to-end gastroduodenostomy) was attempted initially in all patients. When tension was noted at the site of gastroduodenostomy, Billroth II (end-to-side gastrojejunostomy) or Roux-en-Y reconstruction (with 30 cm separating an end-to-side gastrojejunostomy from an end-to-side jejunojejunostomy) was substituted in a random manner. Pathologic stage of gastric cancer, extent of lymph node dissection, and curability were classified according to the Fourth English Edition of the Japanese Classification of Gastric Carcinoma [10] (Table 1). Patients were assigned to group A, B, or C according to the reconstructive procedure (Roux-en-Y, Billroth I, and Billroth II respectively) that they had undergone.

Biopsy from Gastric Remnant

Endoscopic biopsy was performed 12 weeks after surgery in all patients under local anesthesia with lidocaine, using an Olympus videoendoscope (Model XQ-240 or XQ-230; Tokyo, Japan). Three specimens were taken from the lower part of gastric remnant: two for determining the presence of *H. pylori* infection, and one for IL-8 assay.

Table 1. Fourth English Edition of Japanese Classification of Gastric Carcinoma.

Stage grouping	N0	N1	N2	N3
T1	1A	1B	2	4
T2	1B	2	3A	4
T3	2	3A	3B	4
T4	3A	3B	4	4
H1, P1, M1, CY1	4	4	4	4

T1: tumor invasion of mucosa or submucosa; T2: tumor invasion of muscularis propia or subserosa; T3: tumor penetration of serosa; T4: tumor invasion of adjacent structures; N0: no evidence of lymph node metastasis; N1: metastasis of only Group 1 lymph nodes; N2: metastasis of Group 2 lymph nodes, but no Group 3 lymph nodes; N3: metastasis of Group 3 lymph nodes; H1: liver metastasis; P1: peritoneal metastasis; CY1: cancer cells on peritoneal cytology; M1: distance metastasis; D1: dissection of all the Group 1 nodes; D2: dissection of all the Group 1 and 2 nodes; D3: dissection of all the Group 1, 2, and 3 nodes; Curability A: T1 or T2, N0 treated by D1, 2, 3 resection or N1 treated by D2, 3 resection; M0, P0, H0, CY0 and proximal and distal margins > 10 mm; Curability B: no residual disease but not fullfilling criteria for Curability A.

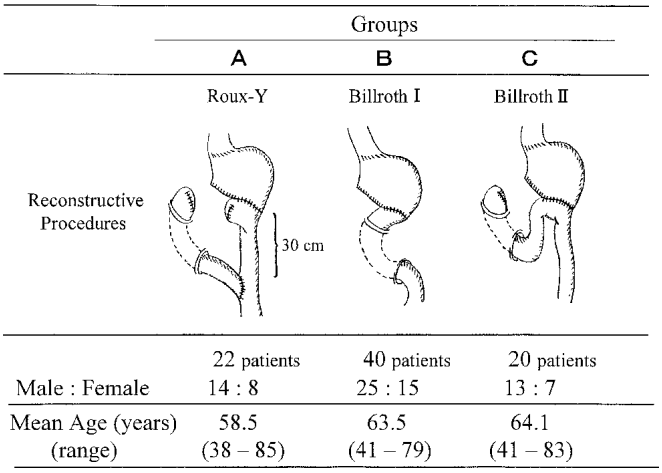


Fig. 1. Reconstructive procedures and patients' profiles.

Assay for IL-8

One biopsy specimen was immediately frozen and stored at −80°C. Samples were homogenized, and aliquots of homogenate supernatants obtained by centrifugation were assayed for total protein by a modified Lowry method. Interleukin-8 concentrations in these supernatants of homogenates were measured by an enzyme-linked immunosorbent assay (ELISA; Research and Diagnostic, Minneapolis, MN) using an immunometric "sandwich" technique. Duplicate samples were incubated in microtiter wells coated with a monoclonal antibody specific for IL-8. Bound IL-8 was detected with peroxidase-conjugated horseradish polyclonal anti-IL-8 antibody. The IL-8 concentration of each sample was derived by comparing the optical density of the sample with a standard curve. This assay is known to accurately measure natural or recombinant endothelial cell-derived IL-8, and it also can predict monocyte reactivity to other cytokines. In our study, the detection limit of this assay is 20 pg/ml, with inter-assay and intra-assay variability of less than 10%.

Diagnosis of Helicobacter pylori *Infection*

One biopsy specimen was fixed in 10% buffered formalin, embedded in paraffin, and sectioned. The presence of *H. pylori* infection was assessed by hematoxylin and eosin staining and by immunostaining using an antibody against *H. pylori* (NCL-HPp, Novocastra, Newcastle-upon-Tyne, UK). In another specimen, urease activity was measured (Helico Check, Otsuka, Tokyo, Japan). When all of these evaluations had a negative result, patients were judged to be uninfected. Patients with at least one positive test, were considered infected.

Statistical Analysis

Interleukin-8 concentrations are expressed as picograms per milligram of supernatant protein. Data are presented as means with standard errors and 95% confidence intervals. Two-tailed Mann-Whitney U tests were used for unpaired comparisons between groups. Comparisons between three groups were performed with a Kruskal-Wallis test. A multi-variant analysis was used for IL-8 concentrations by reconstructive procedures and *H. pylori* infection. Results were considered to be statistically significant when p was less than 0.05.

Ethical Considerations

All procedures including biopsy were carried out in accordance with the Helsinki Declaration. Informed consent was obtained from all patients.

Results

Forty patients (group B) had Billroth I reconstruction, while 22 patients (group A) had Roux-en-Y reconstruction and 20 patients (group C) had Billroth II reconstruction. No difference in age or gender distribution was evident among the three groups (Fig. 1). The pathological stage of disease was Ia in 45 patients; Ib in 14 patients; II in 8 patients; IIIa in 6 patients; IIIb in 7 patients; and IV in 2 patients (Table 2). Stage distribution did not differ in the three groups. All patients had D2 lymph node dissections. To obtain free surgical margin, more than two-thirds of the stomach was resected in all patients. Then most of the parietal cell region was resected. As the result of dissection of cardiac and perigastric nodes, the residual stomach was denervated. Curability after the operation was rated as A or B (Table 1). Intestinal obstruction from adhesions developed in 2 patients in group A and 1 in group B. Minor leakage occurred in 1 patient in group A and 1 patient in group C. All of these postoperative complications subsided with conservative treatment, except in a patient with intestinal obstruction who required surgical intervention.

The prevalence of *H. pylori* infection was 68% (15/22), 48% (19/40), and 45% (9/20) in groups A, B, and C respectively, showing no significant difference between the three groups ($p = 0.22$; Table 3).

The IL-8 concentrations in the gastric remnant were 141.1 ± 21.4 pg/mg protein in patients with *H. pylori* infection and 56.9 ± 11.6 pg/mg protein in patients without *H. pylori* infection ($p < 0.01$; Fig. 2). In patients with *H. pylori* infection, IL-8 concentrations in the gastric remnant were 61.1 ± 11.7 pg/mg protein, 160.6 ± 25.5 pg/mg protein, and 233.6 ± 74.1 pg/mg protein in groups A, B, and C, respectively (Fig. 3). In patients without *H. pylori* infection, IL-8

Table 2. Pathologic stage of gastric cancer.

Pathologic stage	Groups		
	A	B	C
1a	11	24	10
1b	4	7	3
2	3	4	1
3a	1	3	2
3b	2	2	3
4	1	0	1
Total	22	40	20

Staging is based on Japanese Classification of Gastric Carcinoma.
Data are numbers of patients.
There was no difference in stage distribution among the three groups.

Table 3. Incidence of *Helicobacter pylori* infection.

	Groups		
	A (n = 22)	B (n = 40)	C (n = 20)
Helicobacter pylori infection	68.2% (15/22)	47.5% (19/40)	45.0% (9/20)

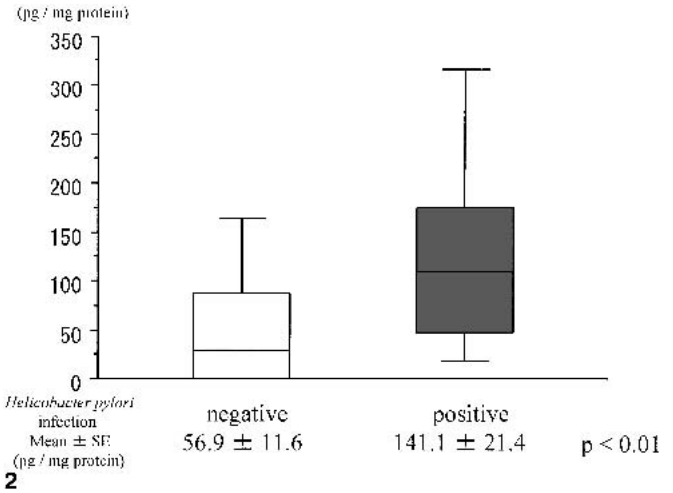
$p = 0.22$.

concentrations in the gastric remnant were 12.7 ± 8.2 pg/mg protein, 55.7 ± 16.6 pg/mg protein, and 87.3 ± 22.6 pg/mg protein in groups A, B, and C respectively (Fig. 3). The differences of IL-8 concentrations were significant ($p = 0.03$; Fig. 3). IL-8 concentrations were significantly higher in patients with *H. pylori* infection than in patients without *H. pylori* infection both in group A ($p = 0.02$) and in group B ($p < 0.001$). In group C, IL-8 in infected and uninfected patients did not differ significantly ($p = 0.10$; Fig. 3).

Discussion

Remnant gastritis is common after distal gastrectomy. Symptoms including dyspepsia, epigastralgia, and heartburn compromise quality of life for patients [3]. Ochiai and Hirohashi [11] immunohistochemically detected a mutant form of p53 protein in biopsy specimens showing chronic atrophic gastritis in 10% of patients who had undergone distal gastrectomy for cancer. In some patients with curative gastrectomy for cancer, such inflammation in the gastric remnant has ongoing potential for carcinogenesis. Nitric oxide (NO) and oxygen radicals induced by inflammation could contribute to this risk [12]. After distal gastrectomy, minimizing remnant gastritis is important for preventing development of carcinoma in the gastric remnant. Duodenogastric reflux is considered a major pathogenetic factor in remnant gastritis [4] and strongly deteriorates the quality of life [13]. In our previous study [9], reconstruction with biliary diversion (Roux-en-Y) after distal gastrectomy proved superior to Billroth I and II reconstructions in preventing development of remnant gastritis. Avoiding remnant gastritis is important for preventing both an additional carcinoma in the gastric remnant and distressing inflammatory symptoms.

Endoscopic examination and pathologic grading of the infiltration by neutrophils in gastric mucosa [5, 6] commonly are used to evaluate remnant gastritis, but both assessments are subjective. Gastrointestinal epithelial cells secrete biologically active IL-8 in response to inflammatory cytokines. Interleukin-8, a peptide secreted predominantly by macrophages, is a potent chemoattractant



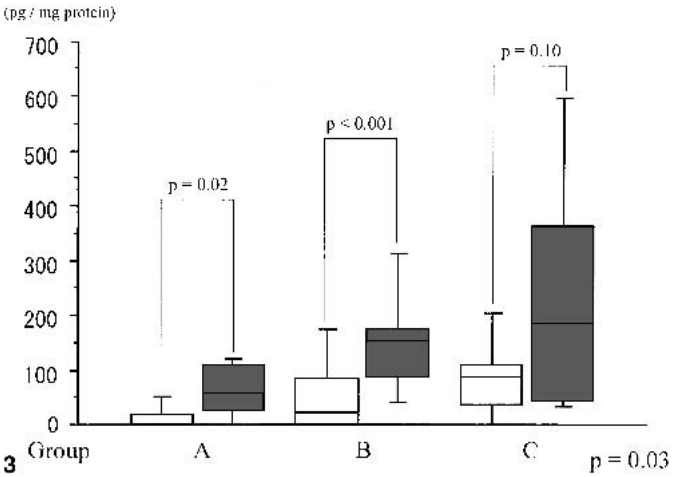


Fig. 2. Interleukin (IL)-8 concentration in the gastric mucosa was significantly higher in patients with than without *Helicobacter pylori* infection.

Fig. 3. IL-8 concentration according to reconstructive procedure as well as presence of *H. pylori* infection. In patients without *H. pylori,* the IL-8 concentration in the gastric mucosa was 12.7 ± 8.2, 55.7 ± 16.6, and 87.3 ± 22.6 pg/mg protein in groups A (Roux-en-Y), B (Billroth I), and C (Billroth II), respectively (open bars). In patients with *H. pylori,* the IL-8 concentration in the gastric mucosa was 61.1 ± 11.7, 160.6 ± 25.5, and 233.6 ± 74.1 pg/mg protein in groups A, B, and C, respectively (filled bars). The differences in IL-8 concentration were significant ($p = 0.03$). In groups A and B, IL-8 concentrations were significantly higher in patients with than without *H. pylori,* but *H. pylori* was not associated with a significant difference in group C.

and activator for neutrophils [14]. Locally infiltrating activated neutrophils are thought to play an important role in development of chronic gastritis and mucosal ulceration, because these cells release reactive oxygen radicals and degradative enzymes [15]. Interleukin-8 in the mucosa of the gastric remnant is a sensitive marker of inflammation that can be measured objectively to evaluate the intensity of remnant gastritis.

Since the first report concerning *H. pylori* by Warren and Marshall [16] in 1983 gastritis, especially gastritis with prominent neutrophil infiltration, has been linked to *H. pylori* infection in the gastric mucosa. Gionchetti et al. [17] reported higher mucosal IL-8 concentrations in patients with than without *H. pylori* infection. In the present study, IL-8 concentrations also were significantly higher in patients with *H. pylori* infection ($p < 0.01$; Fig. 2). These

results reconfirm that *H. pylori* infection increases IL-8 concentrations in gastric mucosa and promotes remnant gastritis.

Interleukin-8 concentrations were significantly lower in group A than in groups B and C, both with and without *H. pylori* infection. We previously have reported that Roux-en-Y reconstruction after distal gastrectomy more effectively prevents duodenogastric reflux than Billroth I or II reconstructions [9]. Preventing duodenogastric reflux is thought to decrease IL-8 concentrations. Interleukin-8 concentrations showed no difference between patients in group A with *H. pylori* infection and patients in group C without *H. pylori* infection. These results indicate that reconstruction with biliary diversion is more effective in reducing remnant gastritis, and presumably risk of carcinogenesis, than other reconstructions. *H. pylori* can be eradicated effectively with antibiotics, if necessary [18].

Résumé. Le rétablissement de continuité selon Billroth I ou II après gastrectomie distale est souvent associé à une inflammation du moignon gastrique. Nous avons cherché à déterminer quel procédé de reconstruction était le plus efficace dans la prévention de la gastrite du moignon. On a comparé trois groupes de patients ayant eu une gastrectomie distale à visée curatrice pour cancer ($n = 82$): groupe A (anse en Y selon Roux, $n = 22$); groupe B (Billroth I, $n = 40$); et groupe C (Billroth II, $n = 20$). On a mesuré les concentrations de l'interleukine (IL)-8 dans la muqueuse gastrique trois mois après chirurgie. En l'absence d'infection par *Helicobacter pylori* les concentrations en IL-8 ont été de 13, 56 et 87 pg/mg de protéines dans les groupes A, B et C respectivement ($p < 0.05$). En présence d'infection *H. pylori*, les concentrations en IL-8 ont été respectivement de 61, de 161 et de 234 pg/mg de protéines dans les groupes A, B et C ($p < 0.01$). Si l'on se réfère aux concentrations en IL-8 dans la muqueuse du moignon, le rétablissement par anse en Y selon Roux après gastrectomie est mieux que les procédés Billroth I et II dans la prévention de la gastrite de moignon.

Resumen. La reconstrucción tipo Billroth I o II luego de una gastrectomía distal con frecuencia se ve asociada con inflamación del remanente gástrico. Nos propusimos determinar qué tipo de procedimiento reconstructivo es más efectivo en cuanto a prevenir este tipo de gastritis del remanente del estómago. Los pacientes sometidos a gastrectomía distal curativa por cáncer ($n = 82$) fueron clasificados como grupo A (Roux-en-Y, $n = 22$), grupo B (Billroth I, $n = 40$) o grupo C (Billroth II, $n = 20$). Se efectuó la determinación de la concentración de Interleuquina (IL)-8 en la mucosa gástrica a los 3 meses de efectuada la cirugía. En ausencia de infección por *Helicobacter pylori,* las concentraciones de IL-8 fueron, 13, 56 y 87 pg/mg de proteína en los grupos A, B y C, respectivamente ($p < 0.05$). En presencia de infección por *H. pylori*, las concentraciones de IL-8 fueron 61, 161 y 234 pg/mg de proteína en los grupos A, B y C respectivamente ($p < 0.01$). La reconstrucción de Roux-en-Y previene mejor la gastritis del remanente que los procedimientos Billroth I y II, a juzgar por las concentraciones de IL-8 en la mucosa gástrica.

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