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Role of *Helicobacter pylori* infection and nonsteroidal anti-inflammatory drug use in bleeding peptic ulcers in Japan

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Background. *Helicobacter pylori* infection and nonsteroidal anti-inflammatory drugs (NSAIDs) are well-known major causes of peptic ulcers. This study aimed to characterize the features of bleeding peptic ulcers in Japan. ***Methods.*** This prospective study evaluated 116 patients revealed to have bleeding peptic ulcers from January 2000 to December 2002. ***Results.*** Eighty-eight of the 116 patients (75.9%) had *H. pylori* infection. Seventy (60.3%) patients were positive for *H. pylori* with no history of NSAID use (group A), and 18 (15.5%) were positive for *H. pylori* with a history of NSAID use (group B). Among the *H. pylori*-negative patients, 15 (12.9%) were associated with NSAID use (group C). Thirteen (11.2%) patients had no *H. pylori* infection or history of NSAID use (group D). Among the 33 patients with a history of NSAID use, 11 were on-demand NSAID users and 14 took daily low-dose aspirin. The patients in groups B and C were significantly older that those in groups A and D, and they more frequently had coexisting diseases compared with group A. In group D, 11 patients had atrophic changes revealed by endoscopic examination, suggesting a past *H. pylori* infection, and these atrophic changes remained at the time of bleeding. Many of the patients in group D had serious comorbidity. Compared with healthy control subjects, the concentrations of both phosphatidylcholine and phosphatidylethanolamine were significantly decreased in the antral gastric mucosa in all patient groups. ***Conclusions.*** NSAID use contributed to bleeding ulcers in 28.4% of patients; thus, low-dose aspirin or on-demand NSAID use may cause bleeding ulcers. There were only two (1.7%) confirmed cases of *H. pylori*-negative, non-NSAID ulcers.

Key words: peptic ulcer, *H. pylori*, NSAID

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Introduction

The discovery of *Helicobacter pylori* has completely changed the etiologic view of peptic ulcers. *H. pylori* is found in more than 90% of patients with duodenal ulcers and in about 70% of those with gastric ulcers, and it is considered to be one of the most important causes of peptic ulcer disease.[1] The use of nonsteroidal anti-inflammatory drugs (NSAIDs) is considered to be another important cause of peptic ulcer disease. It is generally recognized that non-*H. pylori* non-NSAID ulcers are uncommon. Several studies on *H. pylori* infection and the use of NSAIDs among peptic ulcer patients have reported that the frequency of *H. pylori*-negative ulcers ranges from 2% to 11%.[2–5] It has been suggested that bleeding ulcers have a lower association with *H. pylori* infection than nonbleeding ulcers.[6,7]

In Japan, *H. pylori* infects a larger number of people than in Western countries. The incidence of *H. pylori*-negative ulcers without intake of NSAIDs is reported to be very low in the Japanese population.[8] However, the characteristics of bleeding peptic ulcers among Japanese patients have not been fully elucidated. The aim of this study was to clarify the association of *H. pylori* infection and NSAID use with bleeding peptic ulcers in Japan. In addition, we investigated the concentration of phospholipids in the gastric mucosa, because phospholipids play an important role in the protective barrier function of the gastroduodenal epithelium, owing to their hydrophobicity.[9] Our previous study showed that the phospholipid concentration in the gastric mucosa was decreased in patients with peptic ulcers, and the concentration recovered after *H. pylori* eradication.[10]

Patients and methods

This prospective study evaluated patients who were shown to have bleeding peptic ulcers by emergency

endoscopy in our hospital from January 2000 to December 2002. Patients who had a history of *H. pylori* eradication were excluded. Hemostasis was successfully achieved in all patients by endoscopic maneuvers such as a hemoclip or a pure ethanol injection. At 48 h after emergency endoscopy, patients underwent a follow-up endoscopy and complete hemostasis was confirmed. At that time, *H. pylori* infection was confirmed by a rapid urease test (Helicocheck; Otsuka Pharmaceuticals, Tokyo, Japan), using biopsy specimens taken from both the antrum and middle corpus of the stomach. Additional biopsy specimens were taken for the assessment of phospholipid concentrations. Patients who were diagnosed as *H. pylori* negative by the rapid urease test underwent a ^{13}C-urea breath test (UBT) 1 day after follow-up endoscopy. UBT samples were analyzed using a UBiT system (UBiT IR-300; Otsuka Pharmaceuticals), and the cut-off value was less than 2.5‰. Patients identified as *H. pylori* negative after the rapid urease test and UBT were tested for anti-*H. pylori* immunoglobulin G (IgG) using an enzyme-linked immunosorbent assay (J-HM-CAP, Kyowa Medex, Tokyo, Japan). If all three tests were negative, the patient was diagnosed as *H. pylori* negative.

Patients and family members were carefully asked about their use of NSAIDs, including whether they had taken at least one dose of aspirin within 4 weeks before emergency endoscopy. Daily use of NSAIDs was defined as regular intake over 4 weeks. On-demand use was defined as at least one dose within 4 weeks before emergency endoscopy.

Phospholipid determination was performed after informed consent was obtained. Informed consent was obtained from 25 patients in group A (mean age, 52.9 years), nine in group C (mean age, 61.1 years), and seven in group D (mean age, 55.4 years). In addition, the phospholipid concentrations in biopsy specimens from 25 non-*H. pylori* infected non-NSAID using healthy subjects (mean age, 52.4 years) were measured as a control after informed consent was obtained. Healthy subjects without *H. pylori* infection were matched for age and sex with patients in group A (age, 52.4 ± 3.2 years; range, 31–80; men : women, 20 : 5). Biopsy specimens were taken from the greater curvature of the antrum and stored at –80°C until analysis. The stored specimens were homogenized in a tube with 1 ml phosphate-buffered saline, and lipids were extracted as previously described.[11] Phospholipids, phosphatidylcholine (PC), phosphatidylethanolamine (PE), and sphingomyelin (SM), in the lipid extract were measured quantitatively by thin-layer chromatography and flame ionization detection using a latroscan TLC/FID analyzer (Latron, Tokyo, Japan).[12] The phospholipid concentrations were expressed as μg/mg tissue, wet weight.

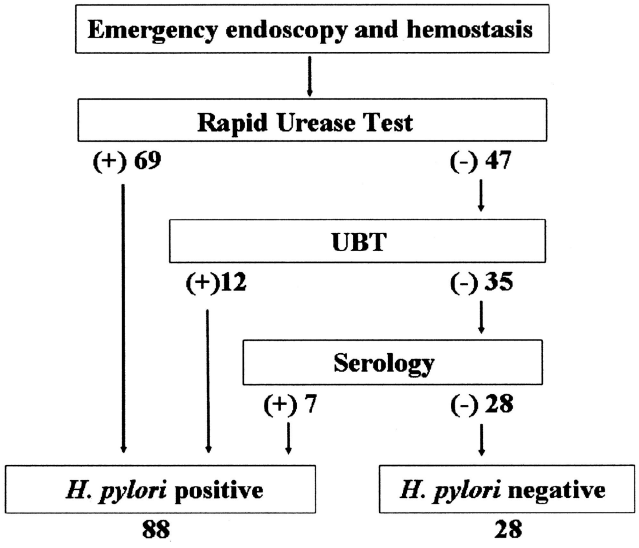

Fig. 1. Diagnostic strategy for *Helicobacter pylori* infection. At follow-up endoscopy, *H. pylori* infection was diagnosed by the rapid urease test for biopsy specimens from both the major curvature of the antrum and middle body of the stomach. Sixty-nine patients were revealed to be *H. pylori* positive by this examination. An additional 12 patients were found to be *H. pylori* positive by the urea breath test (*UBT*). The remaining 35 patients underwent serological examination for anti-*H. pylori* IgG, and seven were positive for the antibody. In all, 88 (75.9%) patients were identified as being *H. pylori* positive and the remaining 28 (24.1%) were *H. pylori* negative

The incidence of non-*H. pylori* and non-NSAID ulcers was compared with that of *H. pylori*- and NSAID-related ulcers. We compared the patients' coexisting diseases and ulcer location. Statistical analyses were performed using one way analysis of variance or the χ-squared test. Differences were considered to be statistically significant when $P < 0.05$.

Results

Between January 2000 and December 2002, we encountered 116 patients who suffered upper gastrointestinal bleeding and were confirmed to have bleeding peptic ulcers. There were 85 (73.3%) men and 31 (26.7%) women, with a mean age of 60.6 years. Ninety-three (80.2%) patients had gastric ulcers, 21 (18.1%) had duodenal ulcers, and two (1.7%) had both gastric and duodenal ulcers. Figure 1 shows the diagnostic strategy for diagnosing *H. pylori* infection. After follow-up endoscopy, *H. pylori* infection was diagnosed using the rapid urease test on biopsy specimens from both the major curvature of the antrum and middle body of the stomach. Sixty-nine patients were revealed to be *H. pylori* positive, and 47 patients were negative. Among

Table 1. Comparison of patients regarding *Helicobacter pylori* infection and nonsteroidal anti-inflammatory drug (NSAID) use

	Group			
	A	B	C	D
H. pylori	+	+	–	–
NSAIDs	–	+	+	–
Number (%)	70 (60.3)	18 (15.5)	15 (12.9)	13 (11.2)
Mean age (years) ± S.E.	58.8 ± 1.9	67.7 ± 4.0*	65.2 ± 5.3*	54.9 ± 4.6
Sex ratio (M:F)	55:15	16:2	4:11*	10:3
Location (stomach:duodenum)	55:15	17:1	11:4	11:2
Daily NSAIDs, no.	–	5	3	–
Low-dose aspirin, no.	–	8	6	–
On-demand NSAIDS	–	5	6	–
Comorbidity, no. (%)	16 (22.9)	17 (99.4)**	14 (93.3)**	7 (53.9)**
Malignancy, no. (%)	6	4	2	5
Death no.	1	0	2	0

Group A, patients positive for *H. pylori* with no history of NSAID or aspirin use; group B, patients positive for *H. pylori* with a history of NSAID and/or aspirin use; group C, patients negative for *H. pylori* with a history of NSAID and/or aspirin use; group D, patients confirmed to be non-*H. pylori* positive and non-NSAID

*Statistically significant compared with group A or group D, $P < 0.05$

**Statistically significant compared with group A, $P < 0.05$

the 47 *H. pylori*-negative patients, 12 were found to be *H. pylori* positive by UBT. The remaining 35 patients underwent serological examination for anti-*H. pylori* IgG, and seven of these were found to be positive for the antibody. In all, 88 (75.9%) patients were identified as *H. pylori* positive, and the remaining 28 (24.1%) were *H. pylori* negative.

Among all 116 patients, 33 (28.4%) had a history of NSAID or aspirin use involving at least one dose within the 4 weeks before emergency endoscopy. Twenty-two patients were daily users, and 11 were on-demand users. Among the 22 daily users, 14 received low-dose aspirin (<100 mg/day).

Table 1 shows the patient profiles and characteristics. Seventy (60.3%) patients were positive for *H. pylori* with no history of NSAID use (group A), and 18 (15.5%) were positive for *H. pylori* with a history of NSAID use (group B). Among the *H. pylori*-negative patients, 15 (12.9%) had a history of NSAID use (group C). Thirteen (11.2%) patients had neither *H. pylori* infection nor a history of NSAID use (group D). The mean age of the patients in groups B and C was significantly higher that that in groups A and D ($P < 0.05$). In group A, 55 (78.6%) of 70 patients were men and 15 (21.4%) were women. In groups B and D, there were more male than female patients. In contrast, in group C with NSAIDs and/or aspirin use but no infection, there were more female than male patients. In group A, duodenal ulcers were relatively common compared with in the other three groups. Comorbidity was lowest in group A. The patients in groups B and C who frequently used NSAIDs and/or aspirin had coexisting diseases ($P < 0.05$), including cardiovascular disease in groups B and C and rheumatoid arthritis in group C.

Table 2 summarizes the characteristics of patients in group D. Seven patients had coexisting diseases (53.85%), and five of these had malignant diseases, which were more evident than in the other groups. Most ulcers were located in the stomach, especially in the upper part of the stomach. We studied the mucosal status by endoscopy and found atrophic changes in 11 of 13 patients. It is suggested that these 11 patients either had had a previous *H. pylori* infection or there was a very small amount of residual *H. pylori* still in the stomach, so that atrophic changes remained at the time of bleeding.

PC, PE, and SM concentrations were measured in patient samples after informed consent was obtained. The results are shown in Table 3. In all *H. pylori*- and NSAID-related and non-*H. pylori* non-NSAID bleeding ulcers, the concentrations of PC and PE were significantly decreased compared with in the healthy control subjects. However, the decreased concentrations did not differ among the three patient groups. The SM concentration was not lower in any of the patient groups.

Discussion

H. pylori infection and NSAID use are two major causes of peptic ulcers. Marshall[1] reported that more than 90% of duodenal ulcers and 70% of gastric ulcers

Table 2. Characteristics of patients in group D

Patient no.	Age/sex	Ulcer location	Coexisting disease	Atrophy
1	64/F	Upper stomach	None	+
2	61/M	Upper stomach	Liver cirrhosis	+
3	74/M	Lower stomach	Malignancy	+
4	55/M	Middle stomach	None	+
5	48/M	Upper stomach	None	+
6	38/F	Upper stomach	SLE	none
7	62/M	Middle stomach	Malignancy	+
8	46/F	Middle stomach	None	none
9	37/M	Upper stomach	None	+
10	27/M	Lower stomach	None	+
11	82/M	Middle stomach	Malignancy	+
12	70/M	Duodenum	Malignancy	+
13	50/M	Duodenum	Malignancy	+

SLE, systemic lupus erythmatosus

Table 3. Phospholipid concentrations in gastric antral mucosa (μg/mg tissue, wet weight)

	Control	Group A	Group C	Group D
Number	25	22	9	7
Age (years, mean ± SE)	52.4 ± 3.2	52.9 ± 2.2	61.1 ± 4.2	55.4 ± 3.1
PC	5.11 ± 0.41	3.41 ± 0.38*	3.09 ± 0.40*	3.78 ± 0.30*
PE	3.22 ± 0.25	2.04 ± 0.22*	1.98 ± 0.29*	2.24 ± 0.19*
SM	0.44 ± 0.10	0.40 ± 0.08	0.38 ± 0.09	0.42 ± 0.11

PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin
* $P < 0.05$

are caused by *H. pylori* infection, and that the most important risk factor for gastric ulcers, other than *H. pylori* infection, is NSAID use. Since that report, several others have reported that the frequency of *H. pylori*-negative peptic ulcers among NSAID users is 2%–11%.[2–5,8] In Australian patients, 6% of duodenal ulcers and 11% of gastric ulcers have been reported to be *H. pylori* negative.[3,4] In the United States, the frequency of *H. pylori*-negative peptic ulcers is reported to be 11%,[2] while the frequencies in Italy and Japan are 4.4% and 1.3%, respectively.[5,8]

It has been suggested that bleeding ulcers have a lower association with *H. pylori* infection than nonbleeding ulcers.[6,7] The most important factor associated with *H. pylori*-negative bleeding duodenal ulcers is NSAID use.[13] In contrast, another study reported that the frequency of non-*H. pylori* non-NSAID bleeding ulcers in Hong Kong is only 4.1%.[14] The authors of that study suggest that underestimation of NSAID use, or unreliable breath urease tests during the acute phase of ulcer bleeding, may be the cause of the high non-*H. pylori* non-NSAID ulcer ratios in previous reports.[14] Other studies have reported that the rapid urease test does not have sufficient sensitivity for use in patients with bleeding ulcers.[15–17] Furthermore, even the breath urease test cannot always detect *H. pylori* infection in bleeding-ulcer cases.[14] The buffering effect of blood in the stomach may contribute to the reduction in sensitivity. Therefore, in addition to the rapid urease and breath urease tests, serological examination and other more sensitive tests should be employed for accurate diagnosis of *H. pylori* infection in patients with bleeding ulcers. We encountered seven patients who were negative for *H. pylori* infection by both the rapid urease test and UBT, but who were subsequently revealed to be *H. pylori* positive by the serum anti-*H. pylori* IgG test.

In this study, we found 13 (11.2%) patients who were negative for both *H. pylori* infection and NSAID use by all three methods of laboratory examination. Their average age was younger than those of the other groups, and the location of the ulcers tended to be in the stomach, with fewer in the duodenum. Many patients had malignancy and other serious coexisting diseases. Chan et al. also mentioned that among their non-NSAID users who had non-*H. pylori* ulcers (4.4%) many had life-threatening diseases such as organ failure or malignancy. Thus, our data are considered to reflect the actual situation in Japan. Malignancy and other

serious diseases may be important risk factors, in addition to *H. pylori* infection or NSAID use. Two patients duodenal ulcers had coexisting malignant diseases that may have contributed to their ulcers. Furthermore, we found that 11 patients had atrophic changes, as revealed by endoscopic examination. Atrophy is usually associated with *H. pylori* infection, and there are reports that gastric atrophy persists even after *H. pylori* eradication.[18,19] Therefore, it is possible that these 11 patients either had had a past infection with *H. pylori* or that only a very small amount of *H. pylori* remained in the stomach, which could not be detected by our examination methods, not even by anti-*H. pylori* IgG. Other more sensitive examinations such as polymerase chain reaction may reveal these patients to be *H. pylori* positive. It is possible that the atrophic membrane itself has an influence on bleeding ulcers. Further examination is required to show whether burn-out atrophy or a very small amount of *H. pylori* can contribute to bleeding ulcers. One of the two group D patients with nonatrophic ulcers suffered from systemic lupus erythematosus and received corticosteroids, which may have caused the bleeding ulcer. We could not find any reason for the bleeding ulcer in one of our patients.

In group A, duodenal ulcers were more common than in the other three groups, and the frequency of coexisting disease was lowest. These features are typical for *H. pylori*-related ulcers and also for nonbleeding ulcers in Japan.[20] In groups B and C, patients with a history of NSAID use had similar characteristics. Their average ages were significantly higher than that of *H. pylori*-related ulcer patients (group A) or of non-NSAID users with non-*H. pylori* ulcers (group D). Duodenal ulcers were rare in groups B and C, but most patients in both groups had some coexisting disease.

The sex ratio was very different between groups B and C. In group B, there were more men than women, whereas in group C most of the NSAID-related ulcers occurred in women. This latter trend has also been reported from England, where NSAID-related ulcers tend to affect older women.[21] This difference may be due to coexisting diseases. Besides the sex ratio, the overall features of group B were closer to those of group C than to those of group A, indicating that NSAID use may be the main cause of the bleeding ulcers in group B. Furthermore, among the 33 patients in groups B and C, 11 were on-demand NSAID users, 14 took low-dose aspirin (<100 mg/day), and only 8 patients were daily NSAID users. This indicates that even low-dose aspirin or on-demand NSAIDs may cause bleeding ulcers in Japan.

Phospholipids in the gastric mucosa play an important role in the protective barrier function of the gastroduodenal epithelium owing to their hydrophobicity.[9] Previous studies have shown that phospholipid concentrations in the gastric mucosa are decreased by *H. pylori* infection.[22,23] We also previously found that the phospholipid concentration in the gastric mucosa is decreased in patients with *H. pylori* infection, and that the phospholipid concentration recovers after *H. pylori* eradication.[10] In the current study, the concentrations of both PC and PE were significantly decreased in all patients, regardless of *H. pylori* infection or NSAID use. There were no differences among the three patient groups. From this observation, we suggest that the mechanism involved in bleeding peptic ulcers is the same regardless of their cause; that is, the etiology is the same for *H. pylori*-related, NSAID-related, and non-*H. pylori*, non-NSAID ulcers.

In the present study, we showed that NSAID use contributed to 28.4% of bleeding ulcers. Low-dose aspirin or on-demand NSAID use can also cause bleeding ulcers. The frequency of non-*H. pylori* non-NSAID bleeding ulcers was 11.6%, including 9.5% of patients with atrophic gastritis, who may have experienced previous *H. pylori* infection or have had a very small, undetectable amount of *H. pylori* still present in the stomach. Patients with confirmed non-*H. pylori* non-NSAID ulcers only represented 1.7% of the total.

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