

Inverse background of *Helicobacter pylori* antibody and pepsinogen in reflux oesophagitis compared with gastric cancer: analysis of 5732 Japanese subjects

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Abstract

Background—The relationship between *Helicobacter pylori* and reflux oesophagitis remains controversial.

Aims—To evaluate the relationship between *H pylori* and reflux oesophagitis in a large number of Japanese subjects.

Subjects—A total of 5732 consecutive Japanese subjects during a health screening were enrolled.

Methods—Gastrointestinal endoscopy was performed on all subjects. We simultaneously measured serum anti-*H pylori* antibody and pepsinogen as markers of *H pylori* infection together with gastric atrophy. The risk of reflux oesophagitis was evaluated in relation to these markers, and the results were compared with those of gastric cancer.

Results—Reflux oesophagitis was found in 108 subjects. Both positivity for *H pylori* antibody (adjusted odds ratio (OR) 0.67 (95% confidence interval 0.45–1.0)) and “low” pepsinogen indicating gastric atrophy (OR 0.35 (0.18–0.68)) were negatively associated with reflux oesophagitis. After subjects were classified into four groups based on positivity or negativity for *H pylori* antibody and “low” pepsinogen, the prevalence of reflux oesophagitis showed a decreasing trend as *H pylori* induced gastric atrophy became more severe. The risk of gastric cancer showed an increasing trend, exactly the opposite to that of reflux oesophagitis.

Conclusions—Analysis of a large series of Japanese subjects revealed a decreasing prevalence of reflux oesophagitis in conjunction with progress of gastric atrophy induced by *H pylori* infection. This pattern was completely opposite to that of gastric cancer cases. A protective role of *H pylori* for reflux oesophagitis through the development of gastric atrophy has been suggested.

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Keywords: *Helicobacter pylori*; oesophagitis; gastro-oesophageal reflux disease; atrophic gastritis; gastric cancer

Although *Helicobacter pylori* has generally been accepted as a pathogen in gastritis, peptic ulcers, gastric cancer, and mucosa associated

lymphoid tissue lymphoma,^{1–4} little is known about the relationship between *H pylori* and reflux oesophagitis. Until recently there were only a small number of studies, and most could find no relationship.^{5–8 9–11} In more recent years, several reports have suggested a protective role of *H pylori* on reflux oesophagitis.^{12–14} It has previously been reported that reflux oesophagitis may develop in duodenal ulcer patients after eradication of *H pylori*^{12 15 16} but subsequent studies reported conflicting results.^{17 18} Gastric atrophy has been suggested to be an important modifying factor for the relationship between *H pylori* and reflux oesophagitis.^{12 14} Several investigators have also pointed to specific strains of *H pylori* as being more protective for gastro-oesophageal reflux disease (GORD).^{13 20} In Western countries, a decrease in the incidence of peptic ulcer and gastric cancer but a remarkable increase in GORD and adenocarcinoma of the oesophagus or cardia^{21–24} have been reported. The decline in *H pylori* infection in these countries has been suggested to be involved in this phenomenon.^{19–21}

The aim of this study was to evaluate the relationship between *H pylori* infection and reflux oesophagitis in a large number of Japanese subjects undergoing medical check ups during a general health screening. This group was considered to be a cross sectional representation of the general Japanese population. We evaluated the background gastric mucosa of reflux oesophagitis cases on the basis of serum *H pylori* antibody and pepsinogen as markers of *H pylori* infection and gastric atrophy.^{25–27} All 5732 subjects underwent endoscopy and measurement of serum markers. The results of the reflux oesophagitis cases were then compared with those of gastric cancer, the association of which with *H pylori* infection has already been established.³

Methods

SUBJECTS

A total of 6489 subjects who received medical check ups, including upper endoscopy, during a regular health screening programme at Kameda General Hospital and Makuhari Clinic were consecutively examined between February 1996 and February 1997. In Japan, these health programmes for healthy individuals are performed in an effort to detect diseases

Abbreviations used in this paper: GORD, gastro-oesophageal reflux disease; OR, odds ratio.

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at an early stage. Patients with specific symptoms requiring prompt medical attention were excluded. As part of their examination, subjects underwent a variety of procedures on one occasion, including physical examination, chest x ray, electrocardiogram, blood laboratory tests, urinalysis, faecal occult blood test, and endoscopy.

After the examination we excluded subjects with peptic ulcers or a history of resected stomach because we considered the possibility of modification to endoscopic findings, *H pylori* status, or pepsinogen levels as a consequence of medication or impaired gastroduodenal function. We also excluded cases of active peptic ulcers and those patients with ulcer scars suggesting healed past ulcers. This excluded group was then evaluated separately.

The protocol was approved by the ethics committees of the respective institutions, and informed consent was obtained from each subject according to the Declaration of Helsinki.

ENDOSCOPY AND CLINICOPATHOLOGICAL CLASSIFICATION

Gastrointestinal endoscopy was performed using electrical panendoscopes (types XQ200 or P230, Olympus, Tokyo, Japan). The bulbar portion of the duodenum, and the entire stomach and oesophagus were carefully observed.

Reflux oesophagitis was defined as visible erosions or ulcerations extending from the gastro-oesophageal mucosal junction, and was classified endoscopically according to the grading system of Savary and Miller.²⁸

Histopathological assessment of gastric cancer was conducted using samples resected by surgical operation or endoscopy. Gastric cancer cases were classified as intestinal or diffuse in pathology using Lauren's classification.²⁹ They were classified as cardiac or non-cardiac in terms of location.

SERUM *H PYLORI* ANTIBODY

Serum anti-*H pylori* IgG antibody was measured using a commercial ELISA kit (GAP-IgG kit; Biomerica INC., California, USA). Seropositivity for *H pylori* antibody was defined by optical density values according to the manufacturer's protocol, and sensitivity and specificity were reported to be 95% and 83%, respectively, compared with results by specific culture.

SERUM PEPSINOGEN LEVEL

Serum pepsinogen was measured using a commercial RIA kit (Pepsinogen I/II RIA BEAD Kit; Dainabot Co., Tokyo, Japan) and served as a marker of gastric atrophy.^{26 27 30-33} It was defined as "low" pepsinogen, possibly indicating gastric atrophy, when the criteria of both serum pepsinogen I level ≤ 70 ng/ml and pepsinogen I/II ratio (serum pepsinogen I (ng/ml)/pepsinogen II (ng/ml)) ≤ 3.0 were simultaneously fulfilled, as proposed by Miki and colleagues.^{34 35} This has been widely applied to mass screening for gastric cancer in Japan on the basis of the hypothesis that atrophic gastritis is a major risk factor for gastric cancer.^{3 33 36} A sensitivity of 70.5% and specificity of 97.0%

for atrophic gastritis compared with histology have been reported in Japan.³⁵

STATISTICAL METHODS

We used SAS software (SAS Institute Inc., North Carolina, USA) for statistical analysis. ANOVA with the Scheffé test was used for intergroup comparisons of mean ages and serum pepsinogen levels. Male to female rates were compared among groups using the χ^2 test. Odds ratios (OR) with 95% confidence intervals (CI) were used as a measure of association, and adjusted for sex and age by unconditional logistic regression models. A two sided p value of less than 0.05 was considered statistically significant.

Results

THE STUDY POPULATION

A total of 6489 Japanese subjects (4319 men and 2170 women, mean age 48.1 years) were examined. Of them, 255 cases with active peptic ulcers (159 gastric ulcers and 108 duodenal ulcers, including 12 with both), 465 with peptic ulcer scars (254 gastric ulcers and 261 duodenal ulcers including 50 with both), and 37 with a resected stomach were excluded from the following analysis.

Hence 5732 subjects (3732 men and 2000 women) were analysed. Mean (SD) age was 48.1 (8.8) years; 905 were <40, 2526 were 40-49, 1685 were 50-59, and 616 were ≥ 60 years old. None of the study subjects had been prescribed proton pump inhibitors, H_2 blockers, or non-steroidal anti-inflammatory drugs within one month prior to the examination. Furthermore, none had undergone eradication therapy for *H pylori*.

REFLUX OESOPHAGITIS AND GASTRIC CANCER CASES

Among the 5732 subjects, 108 cases (93 men and 15 women) of reflux oesophagitis (1.9%) and 26 cases (25 men and one woman) of gastric cancer (0.5%) were detected. The prevalence of reflux oesophagitis was 1.0% (9/905), 2.2% (59/2526), 1.8% (30/1685), and 1.6% (10/616) at ages <40, 40-49, 50-59, and ≥ 60 years, respectively. The prevalence of gastric cancer was 0.22% (2/905), 0.12% (3/2526), 0.65% (11/1685), and 1.6% (10/616) at ages <40, 40-49, 50-59, and ≥ 60 years, respectively.

The grade of reflux oesophagitis was categorised as mild (stage I or II) according to Savary and Miller's classification in all cases. Histopathological features of gastric cancer were considered intestinal in 20 and diffuse in six cases. The gastric cardia was involved in three cases.

SERUM *H PYLORI* ANTIBODY

Among the 5732 subjects, 2695 (47.0%) tested positive for the *H pylori* antibody. The prevalence of seropositivity increased with age, from 34.5% (313/905), 44.6% (1127/2526), 52.4% (883/1685), to 60.4% (372/616) at ages <40, 40-49, 50-59, and ≥ 60 years, respectively.

Table 1 Association of serum *Helicobacter pylori* antibody and serum pepsinogen status with reflux oesophagitis or gastric cancer among all subjects

	Total	<i>H pylori</i> antibody			<i>p</i> Value	Pepsinogen level			<i>p</i> Value
		(+)	(-)	OR (95% CI)		Low	Normal	OR (95% CI)	
Total subjects	5732	2695	3037	1.0	—	1218	4514	1.0	—
Reflux oesophagitis	108	41	67	0.67 (0.45–1.0)	0.05	10	98	0.35 (0.18–0.68)	<0.01
Gastric cancer	26	17	9	1.7 (0.74–3.8)	0.21	16	10	4.2 (1.8–9.6)	<0.01

OR, odds ratio of serum *H pylori* antibody and serum pepsinogen status adjusted for sex and age using unconditional logistic regression models with 95% confidence intervals (CI).

Of the 108 cases of reflux oesophagitis, 41 were positive for *H pylori* antibody. The prevalence of reflux oesophagitis in the *H pylori* antibody (+) and (–) groups was 1.5% (41/2695) and 2.2% (67/3037), respectively (table 1). OR adjusted for sex and age (95% CI) was 0.67 (0.45–1.0; $p=0.05$), showing a negative correlation between *H pylori* antibody and reflux oesophagitis.

Seventeen of the 26 gastric cancer cases were positive for *H pylori* antibody. The prevalence of gastric cancer in the *H pylori* antibody (+) and (–) groups was 0.63% (17/2695) and 0.3% (9/3037), respectively (table 1). Adjusted OR (95% CI) was 1.7 (0.74–3.8; $p=0.21$), showing no significant correlation.

SERUM PEPSINOGEN LEVEL

Among the total number of subjects, 1218 (21.2%) were classified as “low” pepsinogen. The prevalence of “low” pepsinogen increased with age, from 9.6% (87/905), 16.9% (426/2526), 27.2% (459/1685), to 40.0% (246/616)

at ages <40, 40–49, 50–59, and ≥ 60 years, respectively.

Ten of the reflux oesophagitis cases were classified as “low” pepsinogen. The prevalence rates of reflux oesophagitis in the “low” pepsinogen and “normal” pepsinogen groups were 0.82% (10/1218) and 2.2% (98/4514), respectively (table 1). Adjusted OR (95% CI) was 0.35 (0.18–0.68; $p<0.01$), exhibiting a negative correlation between serum pepsinogen level and reflux oesophagitis.

Sixteen of the 26 cases of gastric cancer were classified as “low” pepsinogen. The prevalence rates of gastric cancer in the “low” pepsinogen and “normal” pepsinogen groups were 1.3% (16/1218) and 0.22% (10/4514), respectively (table 1). Adjusted OR (95% CI) was 4.2 (1.8–9.6; $p<0.01$), showing a distinct positive correlation.

Table 2 Characteristics of the four groups categorised by positivity or negativity for serum *Helicobacter pylori* antibody and gastric atrophy determined by serum pepsinogen level

Group	<i>H pylori</i> antibody	Pepsinogen	n	Male	Female*	Age** (mean (SD))	PG I** (mean (SD))	PG I/III** (mean (SD))
A	–	Normal	2703	1767	936	46.0 (8.4)	53.5 (24.4)	6.3 (1.8)
B	+	Normal	1811	1191	620	48.6 (8.5)	72.5 (29.7)	4.2 (1.5)
C	+	Low	884	541	343	51.7 (8.8)	41.7 (17.2)	2.0 (0.7)
D	–	Low	334	233	101	52.3 (9.6)	35.4 (18.5)	1.9 (0.7)

PG I, serum pepsinogen I level (ng/ml); PG I/II, pepsinogen I/II ratio, serum pepsinogen I (ng/ml)/pepsinogen II (ng/ml).

* $p<0.05$ by χ^2 test; ** $p<0.01$ by ANOVA.

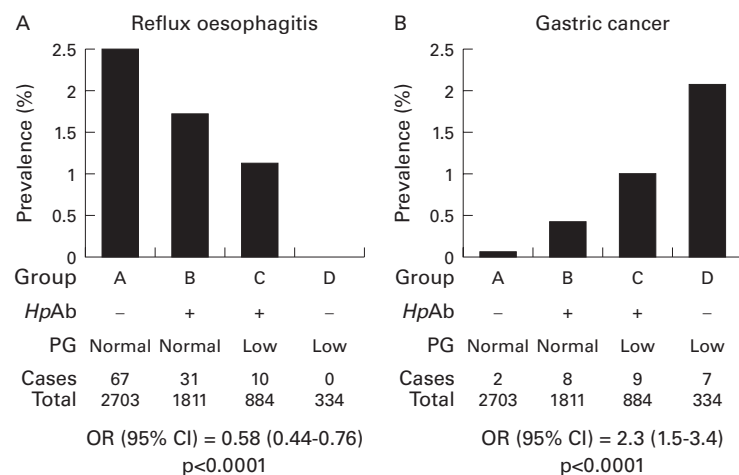


Figure 1 Prevalence of reflux oesophagitis in all subjects in each group, categorised by serum *Helicobacter pylori* antibody (HpAb) and pepsinogen level (PG), showed a gradually decreasing trend in the order of group A to group D (A), the exact opposite to the pattern presented by the increasing trend of gastric cancer (B). The odds ratios with 95% confidence intervals (OR (95% CI)) between adjacent groups, which may reflect the progress of gastric atrophy induced by *H pylori* infection, adjusted for sex and age using unconditional logistic regression models, were 0.58 (0.44–0.76; $p<0.0001$) for reflux oesophagitis and 2.3 (1.5–3.4; $p<0.0001$) for gastric cancer.

ANALYSIS OF THE BACKGROUND OF REFLUX OESOPHAGITIS AND GASTRIC CANCER BY COMBINATION ANALYSIS OF SERUM *H PYLORI* ANTIBODY AND PEPSINOGEN

Subjects were classified into four groups according to positivity and negativity for *H pylori* antibody and gastric atrophy determined by pepsinogen: group A (*H pylori* antibody (–) and “normal” pepsinogen), group B (*H pylori* antibody (+) and “normal” pepsinogen), group C (*H pylori* antibody (+) and “low” pepsinogen), and group D (*H pylori* antibody (–) and “low” pepsinogen). Of the total 5732 subjects, 2703 (47.1%) were allocated to group A, 1811 (31.6%) to group B, 884 (15.4%) to group C, and 334 (5.8%) to group D.

The clinical features of these groups are shown in table 2. Group C showed a significantly lower male/female ratio than the other groups ($p<0.05$ by χ^2 test). Mean ages in groups A to D tended to increase. Serum pepsinogen I level was highest in group B followed by group A, group C, and group D. Serum pepsinogen I/II ratio decreased from group A to group D. Differences in these parameters among groups were all significant ($p<0.01$ by ANOVA).

In the 108 cases of reflux oesophagitis, 67 were classified as group A, 31 as group B, 10 as group C, and 0 as group D (fig 1A). Of the 26 cancer cases, two (including one cardiac cancer) were classified as group A, eight as group B, nine as group C, and seven (including two cardiac cancers) as group D (fig 1B).

As shown in fig 1, the prevalence of reflux oesophagitis among all subjects in each group showed a gradually decreasing trend in the order of groups A to D, a pattern opposite to that of gastric cancer. In reflux oesophagitis

Table 3 Prevalence (%) of reflux oesophagitis and gastric cancer in subjects aged <50 or ≥50 years old

Group	A	B	C	D
<i>H pylori</i> antibody	–	+	+	–
Pepsinogen	Normal	Normal	Low	Low
Reflux oesophagitis				
<50 y	2.3	1.9	1.3	0
≥50 y	2.9	1.5	1.0	0
Gastric cancer				
<50 y	0	0	0.8	1.6
≥50 y	0.2	1.1	1.2	2.4

cases, the *H pylori* antibody (–) and “normal” pepsinogen group showed the highest risk (2.5%, 67/2703) among the four groups, the *H pylori* antibody (+) and “normal” pepsinogen the second highest (1.7%, 31/1811), the *H pylori* antibody (+) and “low” pepsinogen the third highest (1.1%, 10/884), and the *H pylori* antibody (–) and “low” pepsinogen the lowest risk (0%, 0/334) (fig 1A). The order was the exact reverse in the gastric cancer cases, with prevalence rates in each group of 0.07% (2/2703), 0.44% (8/1811), 1.0% (9/884), and 2.1% (7/334), respectively (fig 1B).

Adjusted ORs (95% CI) between adjacent groups were 0.58 (0.44–0.76; $p < 0.0001$) for reflux oesophagitis, revealing a clearly negative correlation, and 2.3 (1.5–3.4; $p < 0.0001$) for gastric cancer, a distinctly positive correlation. To incorporate the parameter of “group” classification into logistic regression models, the serial values of one, two, three, and four were assigned to groups A, B, C, and D, respectively, on the assumption that the association exists in this order. The association with both reflux oesophagitis and gastric cancer was stronger than any other manner of assignment (data not shown).

For fear that the parameter of age in particular might be confounding, the analysis was also performed separately for those <50 and those ≥50 years old. Similar results were obtained in both analyses (table 3).

ANALYSIS OF THE EXCLUDED POPULATION

In the 757 subjects with peptic ulcers, ulcer scars, or resected stomach who were excluded from the analysis, 187 (24.7%) were classified as group A, 421 (55.6%) as group B, 118 (15.6%) as group C, and 31 (4.1%) as group D. The proportion of group B was relatively higher than group A compared with the included population. However, if these subjects had been included, similar results would have been obtained in terms of reflux oesophagitis and gastric cancer (data not shown).

Discussion

Increasing attention has been paid to the relationship between *H pylori* infection and reflux oesophagitis in recent years. The pathogenic role of *H pylori* in reflux oesophagitis was suspected in earlier studies^{37–38} while most other studies found no relationship.^{5–11} In contrast, the possible protective role of *H pylori* in reflux oesophagitis and other GORD related diseases such as Barrett’s oesophagus and oesophageal adenocarcinoma has recently been suggested.^{12–14 20}

Labenz *et al* demonstrated the development of reflux oesophagitis in patients with duodenal ulcers after *H pylori* eradication.¹² However, the latest studies have not always supported these results.^{17 18}

It is clear from this plethora of conflicting evidence that the association between reflux oesophagitis and *H pylori* has yet to be conclusively established, and studies based on large population samples have been conspicuous by their absence. The aim of the present investigation was to shed a definitive light on this problem using a large study population, and to use endoscopic procedures to obtain confirmatory results of reflux oesophagitis.

H pylori is known to evoke gastritis, and chronic gastritis is thought to induce the development of gastric atrophy, metaplasia, and finally cancer.^{3 33–36} Pepsinogen I and II, two main precursors of pepsin, are both produced by the chief and mucous neck cells of the stomach, and were measured as markers of gastric atrophy. Pepsinogen II is also produced by pyloric glands. In mild gastritis, serum pepsinogen I and II levels increase due to inflammation. The increase in pepsinogen II is more prominent, leading to a decrease in the pepsinogen I/II ratio. As chronic gastritis leads to the development of gastric atrophy, the pepsinogen I level specifically decreases due to replacement of chief cells by pyloric glands, and consequently the pepsinogen I/II ratio decreases further. Hence both low serum pepsinogen I level and low pepsinogen I/II ratio are known to be specific markers of gastric atrophy.^{26 27 30–33} In the present study, we attempted to evaluate the status of serum *H pylori* antibody and pepsinogen in patients with reflux oesophagitis, and carried out a comparison with gastric cancer cases.

Our results suggested a negative correlation between reflux oesophagitis and gastric atrophy induced by *H pylori* infection in terms of the status of serum *H pylori* antibody and the level of pepsinogen, in contrast with the positive correlation with gastric cancer. However, both reflux oesophagitis and gastric cancer showed only weak association with *H pylori* antibody which may be explained by the contradictory result that *H pylori* antibody negative subjects included both the highest and lowest risk groups in relation to reflux oesophagitis and gastric cancer.

In this study, group D (*H pylori* antibody (–) and low pepsinogen) showed the lowest risk of reflux oesophagitis and the highest risk of gastric cancer. This is an apparently confusing result if gastric cancer is positively associated with *H pylori* infection.^{3 33–36} Except for group D, groups A–C corresponded to the stages of progressive gastric atrophy induced by chronic infection of *H pylori*, in which those without *H pylori* infection or gastric atrophy (group A) are infected with *H pylori* (group B), and then develop gastric atrophy (group C). Therefore, the characteristics of the *H pylori* antibody (–) and “low” pepsinogen group must be evaluated to ascertain that there exists a previously established association between *H pylori* and gastric cancer.^{3 33 36}

Group D showed the lowest level of pepsinogen I and pepsinogen I/II ratio among the four groups. Previous studies revealed that declines in both pepsinogen I level and pepsinogen I/II ratio were proportional to the extent of gastric atrophy and decrease in acid output.^{26 27 30-33} It has also been suggested that *H pylori* antibody titre might decrease with progression of gastric mucosal atrophy.³⁹⁻⁴² A recent study revealed that in patients with low serum pepsinogen level and histologically confirmed gastric atrophy, *H pylori* antibody titres declined during the mean follow up of 10 years, and some cases actually became negative.⁴³ In our unpublished data, many Japanese patients with chronic atrophic gastritis and negative *H pylori* antibody were finally judged as false negative by the more sensitive gastric juice polymerase chain reaction method.⁴⁴⁻⁴⁶ On the basis of these findings, it is tempting to speculate that this *H pylori* antibody (-) and "low" pepsinogen group simply has a considerably reduced titre of *H pylori* antibody due to the greatly extended gastric atrophy, and this interpretation could well explain the highest risk for gastric cancer in this group. Thus groups A-D reflect the progress of gastric atrophy induced by *H pylori* in this order. Taken together, our results suggest that the extent of *H pylori* induced gastric atrophy is proportionally associated negatively with reflux oesophagitis and positively with gastric cancer, independent of sex or age.

H pylori has been thought to play both pathogenic and protective roles in reflux oesophagitis.^{14 47} Increased gastric acid output (as in duodenal ulcer cases)⁴⁸ and mucosal injury by various cytotoxins due to *H pylori* infection⁴⁹ have been hypothesised as pathogenic mechanisms for reflux oesophagitis. On the other hand, decreased acid output due to extended gastric atrophy^{50 51} and acid neutralising effects of ammonia production by *H pylori*⁵² were reported to inhibit the development of reflux oesophagitis. Our results are not only consistent with the generally accepted notion that *H pylori* induces gastric cancer through gastric atrophy^{3 33 36} but also support the concept that low acid output due to gastric atrophy plays a protective role in reflux oesophagitis.¹²⁻¹⁴

The difference in the distribution of *H pylori* strains between Japan and Western countries could have influenced our results. The CagA positive strain, which is considered more pathogenic for atrophic gastritis,⁵³ peptic ulcers,⁵⁴ and gastric cancer,⁵⁵ was suggested to play a protective role in GORD.^{13 20} Vicari *et al* reported that CagA positive strains decreased in severe complications of GORD such as Barrett's oesophagus and Barrett's dysplasia.¹³ As we have previously shown, most of the *H pylori* strains in Japan are CagA positive.^{56 57} Therefore, our results may specifically indicate an effect of CagA positive *H pylori* strain. In Western countries, the CagA status of *H pylori* strains may be strongly related to outcome.^{13 19 20}

In conclusion, we have shown a gradually decreasing risk of reflux oesophagitis according to the progress of gastric atrophy induced by *H*

pylori infection using a large number of Japanese subjects. This pattern contrasted with that of patients with gastric cancer. This result may suggest a protective role of *H pylori* for reflux oesophagitis, and it may be consistent with epidemiological data from Western countries which show an increase in GORD²¹⁻²⁴ and a decrease in gastric cancer^{21 22} in concert with the decline in *H pylori* infection.¹⁹⁻²¹

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