Methods

The subjects were workers belonging to small- or middle-scale companies who underwent phlebotomy as part of a health check in 1989 and again in 1998. About 9500 workers in 1989 and 8700 workers in 1998 underwent the health checks, of whom 3014 workers underwent health checks in both 1989 and 1998. As *H. pylori* eradication therapy was not paid for by health insurance in Japan before 1999, the subjects did not undergo *H. pylori* eradication during the 9-year period. *H. pylori*-specific immunoglobulin G (IgG) antibodies were measured in residual sera from the health checks in 1989 and 1998 using IgG-GAP™ (Biomerica Co., Newport Beach, CA) according to the manufacturer's instructions. Results were determined as positive, negative or intermediate according to the recommended cut-off values of the test. Sera collected in 1989 had been stored for 8 years at less than −30°C, and sera from 1998 for 1 year at less than −80°C in frozen storage. Seroconversion and seroreversion rates were calculated after excluding subjects with intermediate results in either 1989 or 1998.

Information on the smoking and drinking habits of the subjects was collected in 1989 and in 1998 by questionnaire. The questionnaire in 1998 also asked about abdominal symptoms (epigastric pain, heartburn, fullness of stomach, appetite loss, constipation or other abdominal symptoms) and past history of gastric disease (gastric cancer, gastric ulcer, duodenal ulcer, chronic gastritis or gastric polyps). Relationships between these factors and seroconversion or seroreversion were evaluated. For analysis, subjects were classified into two groups according to smoking habit; that is, those who had continued to smoke and those who did not smoke during the 9-year period. Subjects were also classified into three groups by alcohol consumption; namely, those who continued to drink, those who drank occasionally, and those who did not drink alcohol at all during the 9-year period. For analysis of smoking or drinking habits, those who had changed smoking or drinking habits during the 9-year period were excluded.

Crude relative risks and relative risks adjusted for age and gender were calculated using the χ^2 test and proportional hazard models. Mutually adjusted hazard ratios were then calculated as follows: age, gender and factors giving significant results for seroconversion or seroreversion were put into a proportional hazard model, and a final model was obtained by backward elimination so that all p-values for factors except age and gender were less than 1.0.

Results

Of the 3014 subjects examined, 816 subjects gave intermediate results in either 1989 or 1998 and were excluded from the study. Analyses were carried out using 2198 subjects, whose age and gender distribution is shown in Table 1.

Of the 912 seronegative subjects in 1989, 855 were seronegative and 57 seropositive in 1998. The seroconversion rate during the 9-year period was 6.3%, giving a seroconversion rate per 1000 person-years [with 95% confidence interval (CI)] of 7.0 (5.2–8.7). Of the 1286 seropositive subjects in 1989, 1195 were seropositive and 91 seronegative in 1998. The seroreversion rate during the 9-year span was 7.1%, giving a seroreversion rate of 7.9 (6.3–9.4) per 1000 person-years.

When calculated according to gender, the seroconversion rate was not found to be significantly higher in male subjects, while the seroreversion rate was significantly higher in female subjects. While age was not related to seroconversion rate, seroreversion rates tended to increase with increasing age, with a p-value of 0.08 (Table 1).

Subjects reporting abdominal symptoms or gastric fiberscope use showed a significantly higher seroconversion rate (8.7 vs. 4.5 and 9.2 vs. 1.6, respectively) than controls (Table 2). This result remained statistically significant after adjustment for age and gender (Table 3). Neither dyspepsia nor history of gastric fiberscope use was associated with seroreversion.

Table 1 Seroconversion and seroreversion rates for *H. pylori* antibody during a 9-year period with reference to age and gender

Class	Seroconversion rate[a] (negative in 1989)	Seroreversion rate[a] (positive in 1989)
Total	7.0 (912)	7.9 (1286)
Gender		
Male	7.5 (724)	6.6 (1058)
Female	4.7 (188)	13.6 (228)
p-value	$p = .272$	$p = .001$
Age (years)		
39–44	6.5 (461: 395 + 66)[b]	6.5 (465: 396 + 69)
45–54	6.9 (384: 277 + 107)	8.6 (636: 516 + 120)
55–64	10.7 (52: 40 + 12)	7.8 (156: 125 + 31)
65+	7.4 (15: 12 + 3)	15.3 (29: 21 + 8)
p-value for trend	$p = .385$	$p = .075$

[a]Rate per 1000 person-years.
[b](total: male + female).

Table 2 Factors related to the seroconversion and seroreversion of *H. pylori* antibody during a 9-year period

	Seroconversion	Rate[a] (negative in 1989)	RR1[b] (95%CI)[c]	RR2[d] (95%CI)[c]
Dyspepsia	No	4.5 (373)	1.0	1.0
	Yes	8.7 (539)	1.59 (1.03–2.56)	2.01 (1.12–3.63)
History of duodenal ulcer	No	6.8 (818)	1.0	1.0
	Yes	8.3 (94)	1.02 (0.92–1.13)	1.19 (0.54–2.64)
History of gastric fiberscope	No	1.6 (273)	1.0	1.0
	Yes	9.2 (628)	4.45 (1.81–13.93)	5.35 (1.92–14.90)
Smoking	No	4.8 (390)	1.0	1.0
	Yes	7.5 (386)	1.29 (0.88–1.92)	1.43 (0.74–2.77)
Drinking	No	5.9 (132)	1.0 (p = .465[e])	1.0 (p = .815[e])
	Occasionally	5.5 (142)	0.93 (0.37–2.65)	0.93 (0.33–2.65)
	Yes	7.4 (572)	1.23 (0.56–2.81)	1.07 (0.46–2.49)
	Seroreversion	**Rate[a] (positive in 1989)**	**RR1[b] (95%CI)[c]**	**RR2[d] (95%CI)[c]**
Dyspepsia	No	7.2 (761)	1.0	1.0
	Yes	8.9 (524)	0.88 (0.69–1.11)	0.78 (0.52–1.18)
History of duodenal ulcer	No	8.9 (1032)	1.0	1.0
	Yes	3.5 (254)	0.87 (0.81–0.94)	0.43 (0.21–0.89)
History of gastric	No	8.3 (202)	1.0	1.0
fiberscope	Yes	7.7 (1067)	0.94 (0.58–1.58)	1.08 (0.61–1.92)
Smoking	No	9.2 (640)	1.0	1.0
	Yes	5.4 (454)	0.82 (0.70–0.96)	0.68 (0.40–1.16)
Drinking	No	13.3 (200)	1.0 (p = .001[e])	1.0 (p = .022[e])
	Occasionally	9.0 (223)	0.67 (0.37–1.24)	0.70 (0.38–1.30)
	Yes	5.9 (758)	0.44 (0.27–0.73)	0.52 (0.29–0.91)

[a]Rate per 1000 person-years.
[b]Crude relative risk.
[c]95% confidence interval of relative risk.
[d]Relative risk adjusted for age and gender using proportional hazard model.
[e]*P*-value for trend.

Table 3 Final model using proportional hazard model with backward elimination to assess factors associated with seroconversion of *H. pylori* antibody during a 9-year period

Factors	Hazard ratios (95%CI[a])	*p*-value
Age (+1 year)	1.02 (0.98–1.06)	.435
Gender		
Male	1.0	.399
Female	0.72 (0.34–1.54)	
Dyspepsia		
No	1.0	.047
Yes	1.82 (1.01–3.30)	
History of gastric fiberscope		
No	1.0	.002
Yes	5.08 (1.82–14.16)	

For more details, see text.
n = 901.
[a]95% confidence interval.

Table 4 Final model using proportional hazard model with backward elimination to assess factors associated with seroreversion of *H. pylori* antibody during a 9-year period

Factors	Hazard ratios (95%CI[a])	*p*-value
Age (+1 year)	1.02 (0.99–1.05)	.229
Gender		
Male	1.0	.442
Female	1.24 (0.72–2.14)	
History of duodenal ulcer		
No	1.0	.020
Yes	0.40 (0.18–0.87)	
Drinking (no/occasionally/yes)[b]	0.73 (0.55–0.96)	.026

For more details, see text.
n = 1181.
[a]95% confidence interval.
[b]No, occasionally and yes were defined as 1, 2 and 3, respectively.

Seroconversion was not associated with history of duodenal ulcer, smoking habit or alcohol consumption (Table 2). However, subjects with a history of duodenal ulcer, smoking or alcohol intake showed significantly lower seroreversion rates (3.5 vs. 8.9, 5.4 vs. 9.2 and 5.9 vs. 13.3, respectively) compared to controls. After adjustment for age and gender (Table 2), the significant association with smoking habit disappeared while the associations with history of duodenal ulcer and drinking habit remained. They remained significantly associated with seroreversion even after mutual adjustment (Table 4). All other factors examined showed no significant associations with seroconversion or seroreversion.

Discussion

To avoid overestimation of serochange, subjects with intermediate *H. pylori* antibody titer levels

in either 1989 or 1998 were excluded from the study. If such subjects had been included, spontaneous intrapersonal changes in antibody titers would have been counted as serochange. Therefore, seroconversion and seroreversion rates in this study can be regarded as reliable estimations of real rates of change in *H. pylori* infection status.

Seroconversion

While the overall seroconversion rate was 7.0 per 1000 person-years, the rate was higher in subjects with dyspepsia or a history of gastric fiberscope use. Age and gender were not associated with seroconversion. A Japanese study using the same commercial assay kit and cut-off values for *H. pylori* antibody titers as the present study reported a seroconversion rate of 10.0 per 1000 person-years between 1986 and 1994 [3], similar to our findings in the present study. Similar results have also been obtained in Denmark [9] and in Brazil [10], with seroconversion rates of 9.0 and 11.0 per 1000 person-years, respectively. However, reported seroconversion rates for New Zealand [11], the Netherlands [12] and Italy [13] were lower than in the present study, with values of 1.0, 3.0 and 3.0 per 1000 person-years, respectively, and North American missionaries showed a higher seroconversion rate of 19.0 per 1000 person-years [14]. As developing countries show higher seroconversion rates than developed countries, seroconversion rates in the adult population may depend on the seroprevalence within the population as well as general sanitary conditions. The present study, and another Japanese study, showed similar seroconversion rates to those in developing countries. This may be attributable to the fact that, although sanitary conditions in Japan are similar to those in developed countries, there is a higher prevalence of *H. pylori* in Japan.

The significant association between nonspecific abdominal symptoms and seroconversion was observed even after mutual adjustment. A possible explanation for the significant association is that the abdominal symptoms accompanied the new *H. pylori* infection that caused the seroconversion in some cases. However, it may not be appropriate to connect the abdominal symptoms with seroconversion, because the precise time when the subjects first experienced abdominal symptoms was not obtained in this study.

Although some studies have shown negative results [15], many studies have found *H. pylori* infections to be associated with the use of gastric fiberscopes [16,17]. Guidelines and recommendations for cleaning and disinfecting gastric fiberscopes have been published [18], and were released in Japan in 1995 [19,20]. The observation period of this study (1989–98) encompassed several years prior to the publication of the guidelines, and even after the guidelines were published there has been some debate over whether the gastric fiberscope cleaning and disinfection methods are completely effective [21–23]. Given these considerations, *H. pylori* infection via the gastric fiberscope during the observation period of our study can explain the positive association between seroconversion and gastric fiberscope use. Nevertheless, it is not possible to draw firm conclusions, because the time at which the subjects underwent examinations with a gastric fiberscope was not clear in this study.

Seroreversion

The seroreversion rate in our study was 7.9 per 1000 person-years. This is similar to the rate of 7.0 observed in Denmark [9], higher than the rate of 2.0 reported for Brazil [10], and lower than the rate of 18.0 per 1000 person-years found in another Japanese study [3]. The small number of reported studies makes it difficult to determine the relationship between geographical location and seroconversion rate. In the present study, the seroreversion rate was associated with age, gender, drinking habit and history of duodenal ulcer. It is possible that the seroreversion rate is more dependent on these individual factors than on country or ethnic group, and that it is the distribution of these factors among a population that affects the overall seroreversion rate.

Age showed a weak association with seroreversion. Atrophic gastric mucosa is thought not to be ideal for *H. pylori* [24], and auto-eradication can occur. Atrophy of the gastric mucosa is more frequent and more severe in older subjects [25,26], and may explain the weak association between age and seroreversion. However, this association disappeared after adjustment for gender, drinking habit and history of duodenal ulcer, and the relationship between age and seroconversion, based on the results of our study, remains unclear.

While gender showed an association with seroreversion, this association also disappeared after adjustment for age, drinking habit and

history of duodenal ulcer. Male/female ratios differed depending on age and, as there was a weak positive association between age and seroreversion, it is possible that age was a confounding factor in the relationship between gender and seroreversion. Drinking habit was strongly associated with gender, and drinking habit also showed an association with seroreversion, so that drinking habit may be another confounding factor. Therefore, the apparent association between gender and seroreversion may be an artifact attributable to the presence of confounding factors.

Smoking habit showed an association with seroreversion, which disappeared after age and gender adjustment. However, those with drinking habits or a history of duodenal ulcers showed less frequent seroreversion and both associations remained in the final model. Although few studies have analyzed the relationship between alcohol consumption and seroreversion, a prospective study has shown that seroreversion is rare amongst those with duodenal ulcers [27], which is consistent with the results of our study. A common feature of drinking habit [28,29] and history of duodenal ulcers [30] is that both factors are characterized by increased gastric acid secretion.

Fermented alcoholic beverages containing low or moderate concentrations of alcohol stimulate gastric acid secretion, whereas high alcohol concentrations do not stimulate acid secretion [31]. As consumption of distilled alcohol without dilution is rare in Japan, most subjects with a drinking habit in the present study are expected to have consumed low to moderate concentrations of alcohol, with consequent upregulation of acid secretion. Duodenal ulcer patients show a high prevalence of *H. pylori* infection [32]. The inflammation caused by *H. pylori* infection provokes hyperacidity, which is a cause of duodenal ulcers [33,34]. A recent study revealed that polymorphism of the interleukin-1 beta gene is related to duodenal ulcer risk [35]. As the polymorphism is a genetic factor, those with a history of duodenal ulcers tend be genetically predisposed to upregulated gastric juice secretion.

Increased acid secretion has the effect of diluting and washing off substances contained in food and drink, including substances with bactericidal or bacteriostatic effects. In addition, hyperacidity can inhibit the effect of antibiotics, which is why proton pump inhibitors or H2-blockers are prescribed with antibiotics for the eradication of *H. pylori* [36,37]. Although we do not have any information regarding the antibiotic use of the subjects during the 9-year period, antibiotics are often prescribed for the common cold in Japan. Thus, it is possible that antibiotic use may have exaggerated the inhibitory effect of increased acid secretion on seroreversion.

An inverse relationship between alcohol consumption and *H. pylori* prevalence has been reported in a number of studies [38], including our previous study [39]. The results of the present study suggest that the eradicating effect of alcohol on *H. pylori* infection does not appear to underlie the inverse association observed in cross-sectional studies. Eradication therapies against *H. pylori* in those with drinking habits tend to show higher success rates [40], which at first sight is not consistent with the results of our study. However, as eradication therapies also contain proton pump inhibitors [36] or H2-blockers [37], the presence of these compounds may alter the effect of alcohol intake. Thus, while alcohol consumption may prevent infection with *H. pylori*, it may also inhibit its spontaneous eradication.

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Original article

Trends in the incidence of gastric cancer in Japan and their associations with *Helicobacter pylori* infection and gastric mucosal atrophy

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Abstract

Background. **Although age-adjusted mortality from gastric cancer has been decreasing in Japan, the crude incidence of gastric cancer shows a slight increase.**

Methods. **We have observed trends in the incidence of gastric cancer by sex and 20-year age groups over the past two decades (1976–1996). Source data were obtained from the cancer statistics materials provided by the Research Group for Population-Based Cancer Registration in Japan. Simultaneously, we observed changes in the prevalence of *Helicobacter pylori* infection and in serological atrophy of the gastric mucosa, and compared the results with those involving changes in the incidence of gastric cancer.**

Results. **A slight decline was observed in all age groups over 40 years old, in both men and women, between 1986 and 1996. However, a marked decline in incidence was observed for those aged 20–39 years. The prevalence of *H. pylori* infection declined in both sexes between 1989 and 1998. The frequency of serological atrophy of the gastric mucosa significantly declined in all age groups between 1989 and 1996, with young age groups experiencing a more marked decrease.**

Conclusion. **The marked decline in gastric cancer incidence observed in the young population will also begin to occur in the elderly population in the future.**

Key words Gastric cancer incidence · *Helicobacter pylori* · Pepsinogen · Gastric mucosal atrophy

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Introduction

Despite a marked decline in the incidence of gastric cancer in many industrialized countries, gastric cancer is still the second most common cause of cancer-related deaths in Japan [1–4]. Based on regional cancer registrations, the incidence of gastric cancer in Japan in 1997 was 99318 (male, 66307 and female, 33011), accounting for 20.7% of the total cancer incidence in the same year [5]. The number of gastric cancer deaths in 1997 was 49739 (male, 32218 and female, 17521) based on vital statistics [6]. Age-adjusted mortality has been decreasing in both men and women, although crude mortality has not changed. Gastric cancer mortality is about half of the incidence, due to improved diagnostic and therapeutic techniques.

It is well known that *Helicobacter pylori* infection is one of the major risk factors for gastric cancer, and that low values of the pepsinogen (PG) I-to-II ratio can be a marker for atrophy of the gastric mucosa, as well as a marker for gastric cancer risk [7–10]. Therefore, the prevalence of *H. pylori* infection and the frequency of gastric mucosal atrophy may influence the incidence of gastric cancer.

In this study, we analyzed trends in the incidence of gastric cancer over the past two decades, by sex and 20-year age groups. At the same time, we observed changes in the prevalence of *H. pylori* infection and in serological atrophy of the gastric mucosa, and compared them with changes in the incidence of gastric cancer.

Methods

Cancer incidence figures in Japan are available from the Research Group for Population-Based Cancer Registration of the Ministry of Health, Labour, and Welfare, Japan, which issues annual cancer statistical data. We analyzed trends in the incidence of gastric cancer from 1976 to 1996. The figures from 1985 to 1989 were acquired from the published data [11] that this research group had reported, and the pre-1984 data were from the group's website. The gastric cancer incidence in four 20-year age groups (20–39, 40–59, 60–79, and 80 and over) from 1975 to 1996 was calculated using these data. Mortalities from gastric cancer in 1986 and 1996 were acquired from published data [6] from the Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labour, and Welfare, Japan. To observe trends in gastric cancer incidence and its mortality, the 1996/1986 ratios of incidence and mortality were calculated by dividing the value in 1996 by the one in 1986. In order to compare findings with the results for frequency of serological atrophy of the gastric mucosa, the 1996/1989 ratios of the incidence were also calculated.

For our analysis of the frequency of serological atrophy of the gastric mucosa, subjects were recruited from a workplace in the Kanto-shin-etsu area of Japan; 4486 of the subjects had participated in a health checkup program in 1989, and 4506 had participated in 1996. Serum PG I and serum PG II values were measured using residual sera from the health checkup programs in those 2 years. Measurements were carried out by immunoradiometric assay, with Pepsinogen I/II Riabead kits (Dainabot, Tokyo, Japan). When the serum PG I level was 70 ng/ml or less and the PG I/II ratio was 3.0 or less, it was defined as "serological atrophy" of the gastric mucosa [12].

To determine the prevalence of *H. pylori*, the subjects were selected from workers who belonged to a group of companies in the Tokyo area who were aged 50–59. Six hundred and seventy-six (male, 532; female, 144) of them took part in a general health checkup program in 1989, and 1916 (male, 1580; female, 336) took part in 1998. The sera in 1989 had been frozen for 10 years at −30°C, and those in 1998 had been frozen for 1 year at −30°C before the measurements. Serum *H. pylori* antibodies were measured using both the 1989 and 1998 sera. The measurements were carried out by enzyme-linked immunosorbent assay (ELISA) with Pilika-Plate G Helicobacter II, produced by Biomerica (Newport Beach, CA, USA).

All statistical analyses were conducted using a commercial program for statistical analysis obtained from Halwin Gendai-Sugakusha (Kyoto, Japan). Our studies were approved by the Ethics Committee, Aichi Medical University School of Medicine.

Results

Figure 1 shows changes in gastric cancer incidence by sex in the 20-year age groups. A gradual decline in incidence was observed in all age groups, in both men and women, and the steepest decline was in the 20–39 age group. No clear change was observed before 1986, and a clear decline was observed only between 1986 and 1996, the trend of which was linear and statistically significant in each age group.

The 1996/1986 ratios of gastric cancer incidence for the age groups (Fig. 2) showed that the decline in younger age groups was more conspicuous than that in older age groups. The 1996/1989 ratios gave similar results, but the effect of age was not so clear in the age groups over 40 years.

On the other hand, the 1996/1986 ratios of gastric cancer mortality were in men, 0.516 for those aged 20–39; 0.667 for those aged 40–59; 0.759 for those aged 60–79; and 0.923 for those aged 80 or more. Similarly, in women, the ratios were 0.445, 0.689, 0.652, and 0.849, respectively. The 1996/1986 ratios of gastric cancer mortality showed a trend of decline that was more conspicuous in younger age groups than in older ones.

Figure 3 shows the frequency of serological atrophy of the gastric mucosa in 1989 and 1996. During the 7-year period, the frequency of serological atrophy of the gastric mucosa significantly declined in all age groups, with young age groups experiencing a more notable decrease.

Figure 4 shows the seroprevalence of *H. pylori* among those aged 50–59, in 1989 and 1998. The prevalence of *H. pylori* infection declined significantly in both sexes over the 9-year period of this study.

Discussion

Because the incidence of gastric cancer is low in people under 40 years of age, and because analysis by 20-year groups can maintain the stability of data, we examined the trends in mortality and incidence by 20-year age groups. A decrease in gastric cancer incidence was revealed, with those aged 20 to 40 showing the most rapid decrease, in both men and women. The decrease was observed only after 1986. Highly salted food intake, smoking, and drinking are enumerated as three major risk factors for gastric cancer, other than *Helicobacter pylori* infection and gastric mucosal atrophy. However, these three risk factors do not seem to explain the decrease sufficiently, for the following reasons. Firstly, although a change in the whole amount of salt intake does not necessarily correlate with a change in the dietary intake of highly salted foods, it is unlikely that the dietary intake of highly salted foods fell rapidly, because it

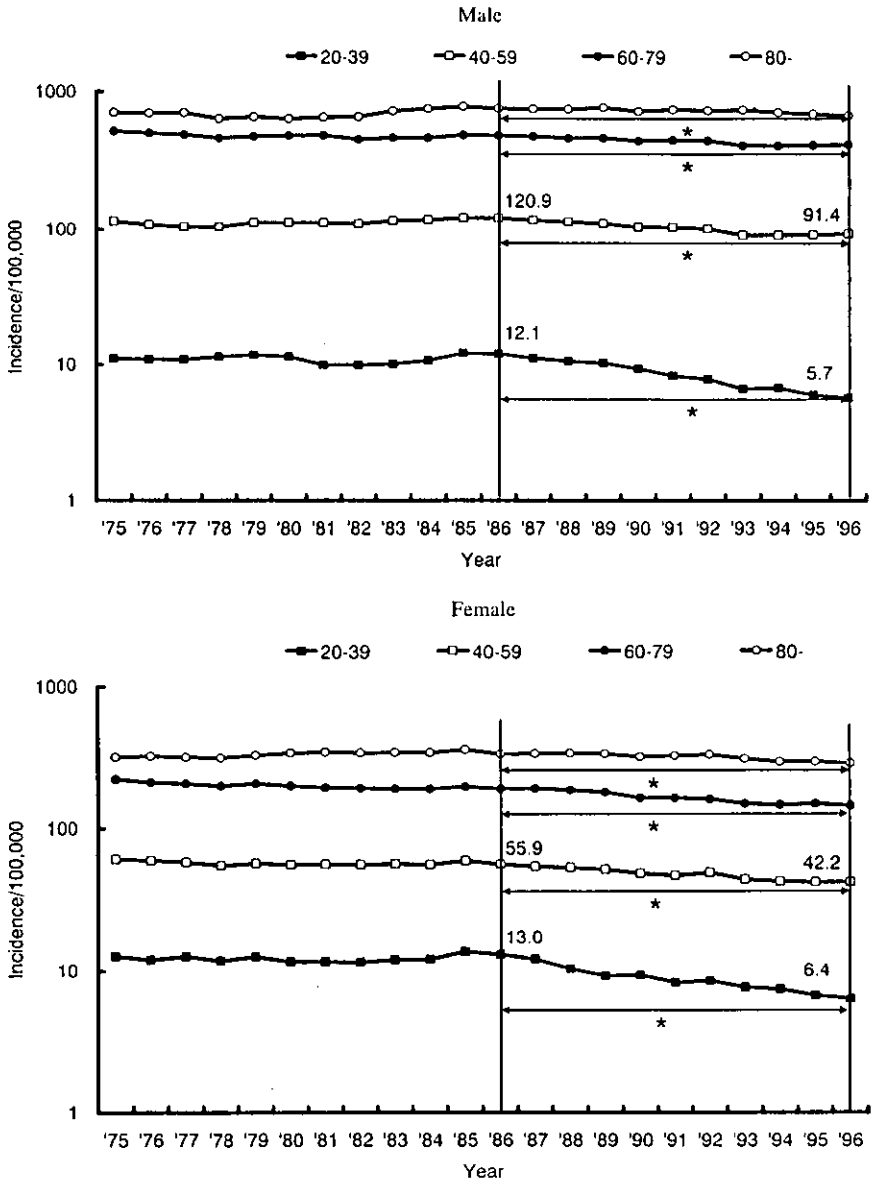

Fig. 1. Changes in gastric cancer incidence according to age are shown. A decline in incidence was observed in all 20-year age groups of both sexes between 1986 and 1996, and the decline in those aged 20–39 years was the steepest. *P*-value for trends, <0.001

has been shown that the amount of salt intake has almost leveled off, according to the National Nutrition Survey in Japan [13]. Secondly, the smoking rate has been decreasing in men and has leveled off in women, but is increasing in young women. The drinking trends have been similar to the smoking trends [14]. Therefore, these three factors do not seem to be major causes of the observed decline in gastric cancer incidence. Thus, among the possible factors contributing to the decrease, two in particular appear to be involved, i.e., changes in the prevalence of *H. pylori* infection, and the diminishing frequency of serological atrophy of the gastric mucosa.

Changes in the prevalence of H. pylori *infection*

Numerous epidemiological and experimental studies using animals have shown that *H. pylori* infection is a causal risk factor for gastric cancer [15,16]. *H. pylori* infection occurs mostly in childhood [17–23] and continues for almost the entire life of the patient. Its overall prevalence is strongly correlated with socioeconomic factors, such as living conditions [24–26], water supply, and sewerage [27,28], which may be especially important in developing countries. In many cross-sectional studies, an increase in the prevalence of *H. pylori* with age has been observed, for which there are two hypotheses: one is that new infections in the aging population increase the gradient of the prevalence; the other is that the gradient is influenced by a cohort effect,

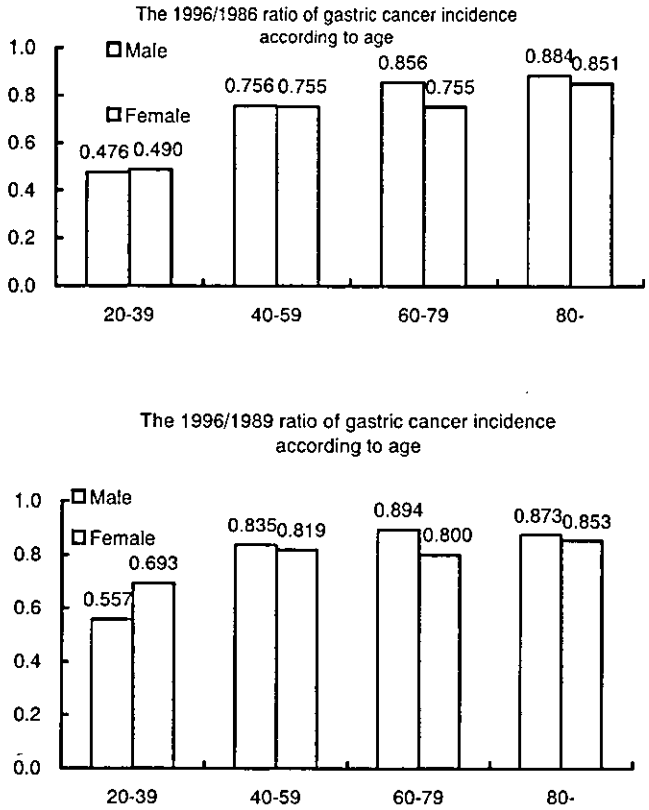

Fig. 2. The 1996/1986 ratios of gastric cancer incidence (over a 10-year period) for each age group showed that the decline was more conspicuous in young age groups than in old age groups. Similar results were observed in the 1996/1989 ratios (over a 7-year period), but the effect of age was not so clear among the age groups aged over 40 years

reflecting more widespread transmission contemporaneous with the childhood years of earlier birth cohorts [19,29,30].

In order to clarify which hypothesis is correct, we compared the prevalence of *H. pylori* between the subjects aged 50–59 years in 1989 and those aged 50–59 years in 1998 in a workers' group and found a decline in seroprevalence over the 9-year period. Although the subjects were only from the Tokyo region, another study with subjects from other areas throughout Japan (Nagano, Niigata, Gunma, Toyama, Shizuoka, Mie, and Miyagi prefectures) has reported the same results [31]. Although these results may not be sufficiently representative of conditions in Japan as a whole, no contradictory results have been found, as far as we can discover, suggesting that the cohort effect of improved hygiene exerts a greater influence on the overall rate of prevalence than new infections with aging [32–34].

Although hygiene conditions in Japan were poor just after World War II, they have gradually improved since about 1950, which seems to support the conclusion mentioned above. If the prevalence of *H. pylori* mainly depends on hygiene conditions in childhood, a decline in *H. pylori* prevalence owing to improved hygiene conditions must occur first in the younger population. These explanations seem to be consistent with the observed decrease in gastric cancer incidence in that young population.

Frequency of serological atrophy of the gastric mucosa

It has been established that people with atrophic gastritis have a high risk for gastric cancer [26,35]. Previous studies have shown that serum PG values were strongly associated with gastric cancer [36]. In people under 40 years of age, the PG II level, as well as the PG I/II ratio, showed a strong association with the risk for gastric cancer [37]. Among those over 40, a strong association was also observed between the gastric cancer risk and serological atrophy of the gastric mucosa [10]. In addition, the relation between atrophic gastritis and gastric cancer has been confirmed by endoscopic studies [38].

Serum PGs are markers for atrophic gastritis and gastric mucosal atrophy. A low serum PG I level and a low PG I/II ratio are related to atrophy of the gastric mucosa, as well as being related to inflammation of the gastric mucosa [39,40]. Therefore, we defined low values for serum PGs (serum PG I level of 70 ng/ml or less and PG I/II ratio of 3.0 or less) as serological atrophy. These low levels are also related to the risk for gastric cancer and are sometimes used in screening programs for gastric cancer [12]. If gastric mucosal atrophy is related to the risk for gastric cancer, a consistent change in the prevalence of gastric mucosal atrophy might be expected in conjunction with the observed decline in the gastric cancer incidence. We observed a change in the frequency of serological atrophy of the gastric mucosa over a 7-year period, and compared it with the change in gastric cancer incidence over the same period.

The frequency of serological atrophy of the gastric mucosa decreased over that 7-year period. The marked decline in frequency of the atrophy cannot be explained without considering the possibility of reversible change in serological atrophy. Indeed, 63% of the subjects experienced an increase or no change in the PG I/II ratio over the 7-year period [39,40], indicating that serological atrophy of the gastric mucosa is reversible.

The decline in young age groups was more conspicuous than that in older groups. Although the subjects did not necessarily represent the Japanese population as a whole, they were, in fact, from relatively wide spread areas: Niigata, Nagano, Gunma, Tochigi, Ibaraki, Saitama, and Tokyo.

So far, many studies have noted the prevalence of gastric mucosal atrophy, but few have examined the

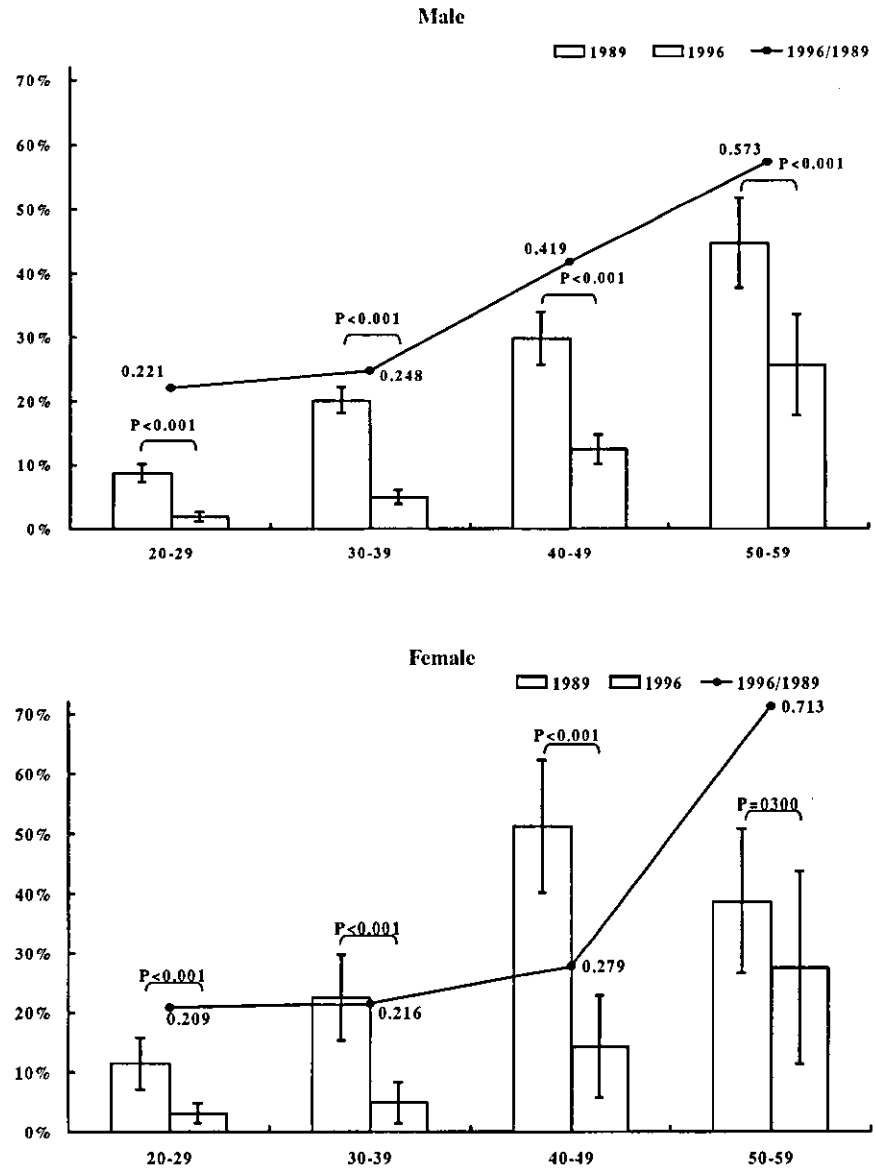

Fig. 3. Frequencies of serological atrophy of the gastric mucosa in 1989 and in 1996. During the 7-year period, the frequency of serological atrophy of the gastric mucosa significantly declined in all age groups, with younger age groups showing a particularly marked decrease

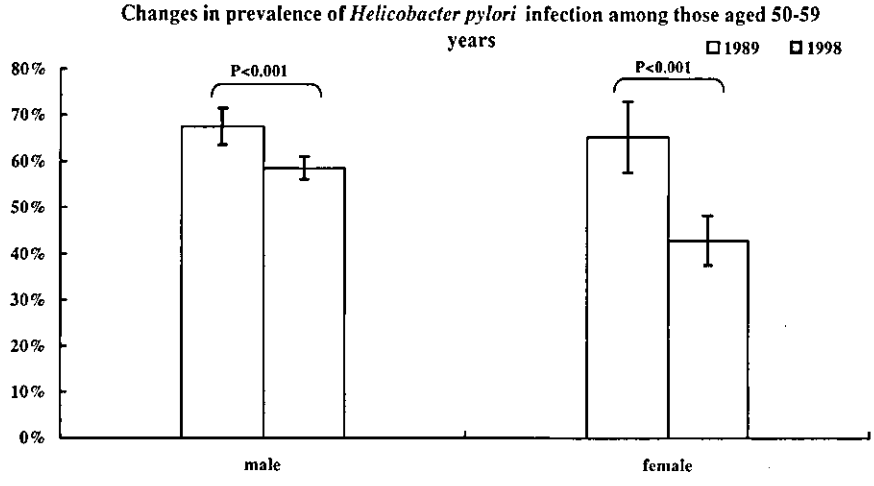

Fig. 4. The prevalence of *Helicobacter pylori* infection declined in both sexes between 1989 and 1998

chronological changes in PG I and II values over a period of several years as our study has. We could find only one study [41] that showed the same trends in serological atrophy as our study did. A large-scale study of Japanese subjects reported that very few cases of atrophy of the gastric mucosa were observed among those without *H. pylori* infection [42], further confirming that a decrease in *H. pylori* prevalence may result in a decrease in gastric mucosal atrophy. Studies reporting a decrease in *H. pylori* prevalence over the years may be considered as indicating a concomitant decrease in gastric mucosal atrophy.

The prevalence of gastric mucosal atrophy seems to have clearly decreased in Japan, along with the prevalence of *H. pylori* infection, with the decrease in the younger population being more conspicuous. Although there may be some limitations to the conclusions discussed above, these findings would seem to explain the decline we observed in gastric cancer incidence. The change in frequency of serological atrophy of the gastric mucosa was more rapid compared with the change in gastric cancer incidence, and this may have occurred because there was a time lag between the beginning of gastric cancer in the atrophic mucosa and the clinical diagnosis of the cancer when it has developed.

Future incidence of gastric cancer

In Japan, there seems to be a decreasing birth cohort effect for *H. pylori* infection, as well as for serological atrophy of the gastric mucosa. The decreasing birth cohort effect is expected to continue in the future. In the observation period of this study, a decline in the incidence of gastric cancer was observed, and it was most marked in those aged 20–39 years. It is expected that the marked decline in the incidence of gastric cancer in the younger population may extend to the older population in the future, as the young population with a low prevalence of *H. pylori* infection gets older.

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□ ORIGINAL ARTICLE □

Comparison of Serum IgA and IgG Antibodies for Detecting *Helicobacter pylori* Infection

Yoshihisa URITA, Kazuo HIKE, Naotaka TORII, Yoshinori KIKUCHI, Hidenori KURAKATA, Eiko KANDA, Masahiko SASAJIMA and Kazumasa MIKI

Abstract

Objective **Although the diagnostic utility of serum IgG antibodies to *Helicobacter pylori* (*H. pylori*) is well established, the usefulness of IgA-based tests is less well documented. The aim of this study was to evaluate two commercially available ELISAs, both for IgG and IgA.**

Patients and Methods **Rapid urease test and histology analysis were performed in 183 patients. A patient was considered to be *H. pylori*-positive when either biopsy test was positive, and considered to be noninfected when both tests were negative. Intestinal metaplasia was determined by dye endoscopy with methylene blue. ELISA testing was performed using the EPI HM-CAP IgG and PP-CAP IgA assays and EIAgen IgG and IgA assays.**

Results **Sensitivity was 94.7, 93.9, 94.8, and 97.0% for HM-CAP IgG, PP-CAP IgA, EIAgen IgG, and EIAgen IgA, respectively. Although sensitivity was excellent for both IgG and IgA antibodies, specificity of both IgA EIAs was low (PP-CAP 72.6%, EIAgen *H. pylori* IgA 59.2%). Three of 101 *H. pylori*-infected patients were PP-CAP positive and HM-CAP negative and four were EIAgen *H. pylori* IgA positive and EIAgen IgG negative. Of eight noninfected patients in whom intestinal metaplasia was found, PP-CAP IgA results were positive in three of five patients with a HM-CAP IgG negative result and EIAgen IgA was detected in one of four patients with an EIAgen IgG negative result.**

Conclusions **Since some patients have IgA positive but IgG negative results, great care should be taken not to underestimate the prevalence of *H. pylori* infection from the results of IgG serology.**

(Internal Medicine 43: 548–552, 2004)

Key words: *Helicobacter pylori*, IgG antibody, IgA antibody, atrophic gastritis, intestinal metaplasia

Introduction

Serological tests are commercially available, easy to perform, and inexpensive and therefore have been recommended for the diagnosis of *Helicobacter pylori* (*H. pylori*) infection (1). Many serological tests, mainly immunoglobulin G (IgG) based, have been validated against invasive methods (2). The IgG antibody level to *H. pylori* is usually increased and may, in assays using specific antigens, be a marker for *H. pylori* infection (3). Because the serum IgG test has shown high sensitivity and specificity, the serology test has been used widely in epidemiologic studies (4, 5). Unlike tests that rely on bacterial urease activity, antibody tests can be performed in patients taking proton pump inhibitors or antibiotics but are not generally useful in the immediate follow-up after eradication therapy (3, 6, 7). Most of these studies have reported a drop in titer at six months of approximately 40–50% from pretreatment levels in patients in whom the bacteria was eradicated (3, 6–9).

Serum antibodies to *H. pylori*, IgG, IgA, and less frequently, to IgM classes, are detected in infected individuals (10). IgM antibodies can be detected shortly after the infection is acquired, but IgA and IgG titers indicate chronic infection (11). Although the diagnostic utility of serologic detection of IgG antibodies to *H. pylori* is well established, the usefulness of IgA-based tests is less well documented. Kosunen et al (10) have described a subset of *H. pylori* infected patients who are positive for IgA but negative for IgG antibodies to *H. pylori*, making the evaluation of IgA titers the only method of serologic confirmation of treatment. The aim of this study was to evaluate two commercially available ELISAs, both for IgG and IgA, for the diagnosis of *H. pylori* infection.

Materials and Methods

Patients

Between October 2000 and March 2001, a total of 183 pa-

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Table 1. Summary of Individual Test Results in *H. pylori*-infected Patients

HM-CAP IgG	PP-CAP IgA	EIAgen IgG	EIAgen IgA	No. of case
+	+	+	+	81
+	−	+	+	2
−	+	+	+	1
+	+	−	+	1
+	−	+	−	1
−	+	−	+	1
+	−	−	−	1
−	−	−	+	2
+	+	Indeterminate	+	2
Indeterminate	+	Indeterminate	+	1
+	+	Indeterminate	Indeterminate	1
−	+	Indeterminate	+	1
Indeterminate	+	+	+	4
Indeterminate	Indeterminate	+	−	1
+	Indeterminate	+	+	1

tients who underwent upper endoscopy for the evaluation of symptoms suggestive of upper gastrointestinal tract disease were evaluated. The patients were 19 to 85 years old (121 women and 62 men), with a median age of 57.7 years. Patients were excluded if they had previously been treated for *H. pylori*, had undergone previous gastroduodenal surgery, or had used a proton pump inhibitor, antibiotics, or bismuth compounds within the previous month.

Endoscopy

After obtaining informed consent, routine upper endoscopy was performed in the usual manner under local pharyngeal anesthesia with the patient lying in a left lateral position. Two antral biopsies were taken from within 3 cm of the pylorus. One antral biopsy was placed in a rapid urease test (RUT, PyloriTek test, Serim Research Corp., Elkhart, IN), the remaining biopsy specimen was fixed with 10% formalin for histologic examination with Giemsa stain. The extension of intestinal metaplasia was judged by dye endoscopy with methylene blue solution. After conventional observation, 20 ml of a 0.5% solution of methylene blue was sprayed on the entire gastric mucosa. Two minutes after the application of methylene blue, approximately 50 to 100 ml of tap water was vigorously sprayed on the gastric mucosa to wash off excess dye. Positive staining which reflected the presence of the columnar mucosa with intestinal metaplasia was defined as blue staining of noneroded mucosa that persisted despite vigorous water irrigation.

A patient was considered to be infected with *H. pylori* when either of two biopsy tests (RUT and histology) was positive, and considered to be noninfected when both tests gave concordant negative results.

Serology

All patients had blood drawn for serological testing at the time of endoscopy. The serum was separated, divided into aliquots, and stored at −20°C before testing. ELISA testing was performed using the EPI HM-CAP IgG and PP-CAP IgA (Enteric Products, Inc., N.Y.) assays and EIAgen *H. pylori* IgG and IgA (BioChem ImmunoSystems, Inc., P.A.) assays. All assays were performed in accordance with manufacturer's instructions. The assays were performed and quantitative Elisa Values (E.V.) extrapolated for each sample according to manufacturer's instructions. Assay values thus calculated for each kit were interpreted as positive, negative, or indeterminate according to the manufacturer's instructions. The calculated ELISA is read as negative if the ELISA value of HM-CAP IgG and PP-CAP IgA is below 1.8, positive if above 2.2, and indeterminate if it is between 1.8 and 2.2. The indeterminate range of EIAgen *H. pylori* IgG and IgA antibody was between 13.5 and 16.5.

Results

Of 183 patients, 101 were *H. pylori* positive and 82 were *H. pylori* negative by using the results of the biopsy tests as the "gold standard". The average age of those infected by the organism was 59.0 years, whereas that for the noninfected individuals was 56.2 years. Among 101 *H. pylori*-infected patients, both RUT and histology were positive in 90 patients. The remaining 11 patients were found to have *H. pylori* infection by RUT alone (n=6) or by histology alone (n=5). Peptic ulcer diseases were detected in 20 patients (14 gastric ulcers, six duodenal ulcers); all of them were *H. pylori* infected. Two of the infected patients had gastric cancers.

Summary of individual results is shown in Tables 1 and 2. In 81 of 101 *H. pylori*-infected patients, all the tests provided the positive results (Table 1), whereas in only 33 of 82 noninfected patients all four tests were negative (Table 2). Discrepancy between PP-CAP IgA and HM-CAP IgG was found in 27 of 160 patients (16.9%) when patients with at

Table 2. Summary of Individual Test Results in Noninfected Patients

HM-CAP IgG	PP-CAP IgA	EIAgen IgG	EIAgen IgA	No. of case
–	–	–	–	33
–	–	–	+	7
+	–	–	–	1
+	–	–	+	2
–	+	–	+	8
–	+	+	+	2
+	–	+	+	1
+	+	+	+	4
Indeterminate	–	–	–	5
Indeterminate	+	+	+	1
Indeterminate	+	–	+	1
+	Indeterminate	+	Indeterminate	1
–	Indeterminate	–	+	3
–	Indeterminate	–	–	3
–	Indeterminate	–	Indeterminate	2
Indeterminate	+	–	+	1
–	+	–	Indeterminate	4
–	–	–	Indeterminate	4

Table 3. Summary of HM-CAP/PP-CAP and EIAgen *H. pylori* IgG/IgA Results Compared with Results of RUT and Histology

	Indeterminate Results	True Positive	Fale Positive	True Negative	False Negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
EIAgen IgG	5	91	9	73	5	94.8	89.0	91.0	93.6	92.1
EIAgen IgA	12	97	29	42	3	97.0	59.2	77.0	93.3	81.3
HM-CAP IgG	13	90	9	66	5	94.7	88.0	90.9	93.0	91.8
PP-CAP IgA	11	93	20	53	6	93.9	72.6	82.3	89.8	84.9

least one indeterminate result were excluded.

The overall sensitivity, specificity, and positive and negative predictive values are given in Table 3. The HM-CAP IgG titers were above the cut-off value in 90 of 101 *H. pylori*-infected patients and in 9 of 82 noninfected patients. PP-CAP IgA results were positive in 93 of 101 *H. pylori*-infected patients and in 20 of 82 noninfected patients. EIAgen IgG and IgA antibodies were detected in 91 and 97 of the 101 *H. pylori*-infected patients and in 9 and 29 of the 82 noninfected patients, respectively. Sensitivity was 94.7, 93.9, 94.8, and 97.0% for HM-CAP IgG, PP-CAP IgA, EIAgen IgG, and EIAgen IgA, respectively. Although sensitivity was excellent for both IgG and IgA antibodies, specificity of both IgA ELISAs was low (PP-CAP 72.6 %, EIAgen *H. pylori* IgA 59.2 %). Three of 101 *H. pylori*-infected patients were PP-CAP positive and HM-CAP negative and four were EIAgen *H. pylori* IgA positive and EIAgen IgG negative. In such cases, determination of specific IgA was informative, giving positive results, while the IgG titers were less than the cutoff value. Accuracy was higher for IgG ELISAs than for IgA ELISAs because of lower specificity of IgA.

Of eight noninfected patients in whom intestinal metaplasia was found by dye endoscopy, PP-CAP IgA results were positive in three of five patients with a HM-CAP IgG negative result and EIAgen IgA was detected in one of four patients with an EIAgen IgG negative result (Table 4).

Discussion

Serology is the noninvasive technique of choice to detect *H. pylori* infection because it is simple, widely available, and inexpensive. The reported sensitivity and specificity of IgG serology is highly variable, ranging from 30% to 100% (12–14). The HM-CAP IgG, evaluated in our study, had a sensitivity, specificity, positive predictive value, and negative predictive value of 94.7, 88.0, 90.9, and 93.0%, respectively. These values were very similar to those of EIAgen IgG. False-positive results of HM-CAP IgG and EIAgen IgG were obtained in 9 patients. When we compared the sensitivity and specificity of serology against biopsy-based methods such as histology and rapid urease test, defined as the gold standard, failure of the biopsy methods to detect the organism may decrease the serological true-positives and increase the false-positives. False-negative biopsies could occur when the active site of infection was missed because of the patchy

Table 4. Results of Serological Tests in Eight Patients with Extensive Intestinal Metaplasia in Whom *H. pylori* Infection was Determined to be Negative by RUT and Histology

No	Age	Gender	EIAgen IgG	EIAgen IgA	HM-CAP IgG	PP-CAP IgA
1	73	F	–	Indeterminate	–	+
2	67	M	+	+	–	+
3	76	F	–	–	–	–
4	73	F	+	+	+	+
5	78	M	–	+	–	+
6	79	F	+	+	+	+
7	70	F	+	+	+	+
8	76	F	–	–	–	–

distribution of *H. pylori* in the stomach. Multiple biopsy specimens from different areas of the stomach may reduce sampling errors.

In contrast, patients who have had a previous infection with *H. pylori* and whose antibody levels were still elevated may have a negative histology and a positive result of serology. Despite the presence of serum antibodies, failure to detect *H. pylori* in biopsy specimens could have been due to atrophy or intestinal metaplasia of gastric mucosa (14, 15). It has already been reported that this may be a major problem in the elderly (16). Some elderly patients with a negative IgG result may have been infected previously and seroreversion may occur, reflecting that the organism have been eradicated spontaneously by the progression of atrophic gastritis and intestinal metaplasia. Based on these viewpoints, dye endoscopy was performed to evaluate the presence of intestinal metaplasia which was considered to relate to *H. pylori* infection.

Intestinal metaplasia was found significantly more often in the *H. pylori*-positive group than in the *H. pylori*-negative group. Japanese previous reports (17, 18) indicated that the prevalence of IgG antibodies to *H. pylori* was lower in the elderly people compared with those less than 40 years of age. It has been unclear whether the low prevalence of IgG antibodies to *H. pylori* in the elderly reflects spontaneous eradication of the organism. Miwa H et al (19) demonstrated insufficient diagnostic accuracy of imported serological kits for *H. pylori* infection in the Japanese population. However, several studies even in Western countries reported that the accuracy of serological tests in the elderly is unsatisfactory (16).

On the other hand, several studies supporting the clinical utility of IgA serology have appeared. Two studies have noted few patients with confirmed *H. pylori* infection and with only IgA antibodies (20, 21). Aromaa A et al (22) reported that IgA antibodies and low levels of pepsinogen I increase the risk of gastric carcinoma. In addition, IgA antibodies may appear earlier than IgG antibodies in patients who become reinfected (8, 22).

While the sensitivity of all four tests was good the present study, with values above 92%, specificity was very low for the two IgA-based tests. In general, one can expect an IgA ELISA to have lower sensitivity values than an IgG ELISA because most individuals exhibit a predominantly IgG immune response to infection with *H. pylori* (23). However, some investigators have found that about 2% of patients produce an IgA response in the absence of an IgG response (10, 20). Furthermore, Jaskowski et al (24) showed a higher frequency of IgA-positive IgG-negative patients (38/824 cases; 7.2%) with gastrointestinal disorders suggestive of *H. pylori* infection. It is suggested that *H. pylori* infection is excluded by the clinician in the majority of these infected patients solely on the basis of a negative IgG serology result. In our population, three (3.0%) of 101 infected patients was IgA positive and IgG negative. Of eight noninfected patients in whom intestinal metaplasia was found by dye endoscopy, PP-CAP IgA and EIAgen IgA results were positive in three of five patients with a HM-CAP IgG negative result and in one of four patients with an EIAgen IgG negative result, respectively. Since positive serology results are evidence of contact with *H. pylori* but do not necessarily indicate current infection, determination of IgA is informative for such cases. Furthermore the prevalence of atrophic gastritis and intestinal metaplasia is very high in *H. pylori* positive Japanese (17). In conclusion, great care should be taken not to underestimate the prevalence of *H. pylori* infection from the sesults of IgG serology in clinical practice.

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Alimentary Tract

Breath sample collection through the nostril reduces false-positive results of ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* infection

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Abstract

Background. One of the disadvantages of ^{13}C-urea breath test is possible interference by urease activity not related to *Helicobacter pylori*.
Aims. We design the simple and non-invasive modification to avoid the contamination of $^{13}CO_2$ produced in the mouth.
Patients and methods. One hundred and twenty-nine patients who underwent diagnostic upper endoscopy were enrolled. Within 1 week of the endoscopic procedure, each patient received the modified ^{13}C-urea breath test. Breath samples were collected at baseline and at 1, 3, 5, 10, 15, 20 and 30 min after ingestion of 100 mg ^{13}C-urea solution through the mouth and the nostril at each time point.
Results. The breath $\Delta^{13}CO_2$ value through the nostril at 1 min was already higher in *H. pylori*-positive patients than in *H. pylori*-negative patients. Using 2.5‰ as the cut-off value, the sensitivity and specificity of the modified ^{13}C-urea breath test at 20 min were both 100%, whereas the sensitivity and specificity of the standard ^{13}C-urea breath test were 97.7 and 94%, respectively, using 3‰ as the cut-off value.
Conclusions. The modified ^{13}C-urea breath test in which breath samples are collected through the nostril provides an easy way of avoiding false-positive results for the detection of *H. pylori* infection.

Keywords: ^{13}C-urea breath test; *H. pylori*; Sample collection through the nostril

1. Introduction

^{13}C-urea breath test (UBT) has become the most convenient non-invasive method for the diagnosis of the presence of *H. pylori* infection [1–4]. One of the main disadvantages of UBT is possible interference by urease activity not related to *H. pylori*, as there is bacterial flora in the mouth and the intestine. In order to eliminate the problem of false-positive results in early breath samples, due to urease-producing bacteria other than *H. pylori*, some modifications of UBT have been suggested, such as mouth washing [5,6], or supplying ^{13}C-urea as a rapid-release tablet [7,8]. A shorter time of breath sample collection may also be important for diagnostic value, especially for persons with rapid gastric emptying, and for avoiding false-positive results from the rapid transit of ^{13}C-urea to the colon. An endoscopic UBT, in which ^{13}C-urea solution is sprayed directly into the stomach through a biopsy channel, is one of the useful modifications to avoid the contamination of $^{13}CO_2$ produced in the mouth and the intestine [9–11]. Despite an endoscopic UBT has high diagnostic reliability for the diagnosis of *H. pylori* infection, they are invasive and stressing for the patient. In the present study, we design the more simple and non-invasive modification of UBT.

2. Patients and methods

One hundred and twenty-nine patients who underwent diagnostic upper endoscopy for gastrointestinal symptoms were enrolled in the present study, including 75 females and 54 males, with a mean age of 60.3 (14–79) years. None of

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129 patients enrolled had symptoms such as nose obstruction or nose discharge. To avoid impediments in UBT results, patients were excluded initially due to the presence of any of the following conditions: the ingestion of proton pump inhibitors, H_2-receptor antagonists, antibiotics, or bismuth salts in the previous 2 months; previous gastrointestinal surgery; and having a past history of *H. pylori* eradication therapy. The study was approved by our local ethics committee, and prior written consent was given by all patients included in this study.

At endoscopy, after noting the presence and location of abnormal findings, two biopsy specimens were taken from two sites on the greater curvature of the antrum and the midbody of the stomach. The biopsy specimens were placed in 10% buffered formalin fixative for routine processing, sectioning and staining with haematoxylin and eosin and Giemsa stains. At least one experienced histopathologist, who was blinded to endoscopic findings, evaluated the specimens. *H. pylori* was determined by Giemsa-staining sections. In addition, *H. pylori* IgG antibody concentrations were measured with an ELISA method (HM-CAP). A value of >2.2 was considered positive and a value of <1.9 was considered negative. Patients with a value of not less than 1.9 nor more than 2.2 were excluded in this study. Patients with positive *H. pylori* histology were considered *H. pylori*-positive. Both histology and serology were required to be negative to establish a patient as being without *H. pylori* infection. Patients with positive antibody and negative histology were excluded from analysis.

Within 1 week of the endoscopic procedure, each patient received the modified UBT. After overnight fasting, 100 ml tap water and 100 mg ^{13}C-urea solution were used. Breath samples were taken at baseline and at 1, 3, 5, 10, 15, 20 and 30 min after administration. At each time point breath samples were collected in duplicate through the mouth and the nostril. After an approximately normal inspiration and a 15-s breath-hold, the patient squeezed one nostril with their second finger and placed a sampling port of collection bag into another nostril, forming a tight seal around it with the nostril (Fig. 1). They blew into a collection bag through the nostril like blowing their nose. Although the collection bag used had a one-way breathing valve, using the cap plug assured that sample volume would not be lost due to a leak through the flap-valve. As soon as the collection bag was removed from the nostril, a new one was placed in the mouth and a breath sample was collected through the mouth. These breath samples were analysed on isotope ratio mass spectrometer (ABCAG: Europa Scientific, Crewe, UK), which measures the ratio of the heavy and light isotopes in a sample and compares this to a standard gas. The $^{13}C/^{12}C$ ratio was calculated and expressed as delta over baseline ($\Delta‰$).

The cut-off values of the UBT at each time point were calculated separately according to the sensitivity, specificity and accuracy. The optimal cut-off value of excess $\Delta^{13}CO_2$ for each protocol was determined by the accuracy. The McNemar's test was used to assess statistical differences among sensitivity and specificity of different UBTs. In a time course study, the differences of mean $\Delta^{13}CO_2$ values at each time point between modified UBT and standard UBT were assessed with Student's paired *t*-test. A P value of <0.05 was considered significant.

Fig. 1. Schematic drawing showing the breath sample collection technique through the nostril. After an approximately normal inspiration and a 15-s breath-hold, the patient squeezed one nostril with their second finger and placed a sampling port of collection bag into another nostril, forming a tight seal around it with the nostril. They blew into a collection bag through the nostril like blowing their nose.

3. Results

Two patients were excluded from analysis because they had positive serology and negative histology. Of the remaining 127 patients, 42 had *H. pylori* infection. When using the standard UBT, in which breath samples were collected through the mouth, the breath $\Delta^{13}CO_2$ values at 1 min after ingestion of ^{13}C-urea solution did not differ between *H. pylori*-positive patients and *H. pylori*-negative patients (Fig. 2). At 3 min and all subsequent time points, however, the breath $\Delta^{13}CO_2$ values were significantly higher in *H. pylori*-positive patients, compared with those in *H.*