

ORIGINAL ARTICLE

Relative risk of gastric cancer between those with and without *Helicobacter pylori* infection history in Japan

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Abstract

Background and Aim: The causal relationship between *Helicobacter pylori* (*H. pylori*) infection and gastric cancer has been established. Although the magnitude of the carcinogenic effect of *H. pylori* is the next concern, it has not been sufficiently evaluated in Japan. Spontaneous disappearance of *H. pylori* infection may have provoked underestimation of the carcinogenic effect of the infection. To reduce the influence, a comparison should be carried out between subjects with and without the infection history. Cutoff values of *H. pylori* antibody lower than the manufacturer's recommendation are known to be more appropriate to diagnose history of *H. pylori* infection. The aim was to evaluate the carcinogenic effect of *H. pylori*.

Methods: A case-control study consisting of 275 gastric cancer patients and 275 age- and sex-matched controls was performed. Serum *H. pylori* antibody was measured using the "JHM-Cap" kit with a domestic antigen (cut value of the manufacturer's recommendation was 2.3 EV: ELISA value). Using a conditional logistic model, the odds ratios (ORs) for five cutoff values adjusted for smoking and drinking doses were calculated.

Results: For cutoff values of 1.25, 1.5, 1.75, 2.0, and 2.3 EV, the ORs (95% confidence intervals) were 67.7 (9.1, 502), 37.2 (8.8, 157), 21.3 (9.0, 60.2), 25.5 (9.0, 72.7), and 25.9 (9.2, 73.2), respectively.

Conclusions: These results suggest that the risk ratio of gastric cancer between subjects with and without history of *H. pylori* infection in Japan may exceed 20.

Introduction

In Japan, gastric cancer has been one of the major cancers; however, its incidence and mortality have been decreasing recently.¹⁻³ *Helicobacter pylori* is known to be a strong carcinogen of gastric cancer.⁴ To date, epidemiological studies have evaluated the relationships between *H. pylori* and the risk of gastric cancer worldwide,⁵⁻⁷ including in Japan,⁸⁻¹⁰ and showed the relationship is causal. The next concern is the magnitude of the carcinogenic effect of *H. pylori*. The magnitude is inevitable to make gastric cancer prevention programs more effective.

There are many *H. pylori* strains in the world, and the differences in their virulence result in the differences in gastric cancer incidence depending on geographic area.¹¹⁻¹³ Therefore, the magnitude of the carcinogenic effect of *H. pylori* should be evaluated in each area.

Although gastric cancer risk is reduced to around two-thirds after successful eradication of *H. pylori*,¹⁴ the risk keeps a level that is much higher than in those without *H. pylori* infection history for at least 20 years.¹⁵ Gastric cancer risk in those after the spontaneous disappearance of *H. pylori* is almost the same as or sometimes higher than those with current infection.¹⁶ These facts indicate that *H. pylori* may have completed most of its role

as a gastric carcinogen until the time of the spontaneous disappearance.

Since atrophic gastritis is a positive risk factor of gastric cancer,¹⁷ atrophic gastritis is more frequent in gastric cancer patients than in other subjects. The surface of gastric cancer tumor is uncomfortable for *H. pylori*.¹⁸ Therefore, spontaneous disappearance of *H. pylori* occurs in gastric cancer patients more frequently than in other subjects.^{9,19} Thus, to estimate the precise carcinogenic effect of *H. pylori*, comparisons should be performed not between those with and without infection at baseline or at diagnosis, but between those with and without a history of infection.

After loss of infection, serum antibody titer gradually decreases to reach the similar level in those without history of the infection.^{20,21} To diagnose subjects who experienced loss of the infection as high risk, it has been recommended to lower the cutoff values of *H. pylori* antibody.²²⁻²⁴ Therefore, a cutoff value of serum *H. pylori* antibody titer lower than the manufacturer's recommendation may be more appropriate to diagnose history of *H. pylori* infection.

We reanalyzed the data of our previous case-control study,²⁵ where a *H. pylori* antibody kit with a domestic antigen

and detecting antibody keeping a high titer for a relatively long time was used.²⁶ The odds ratios (ORs) were calculated with several cutoff values: the manufacturer's recommendation and the lower ones.

Since the incidence of gastric cancer is not high, its OR is almost the same as its risk ratio (RR). In this study, RR is used in two ways: to present the results of cohort studies and as a marker representing the magnitude of influence of *H. pylori* infection.

Methods

The subjects were the same as those of our previous study,²⁵ who were selected from our original case-control study with

788 gastric cancer patients and 1007 controls.⁹ Briefly, cases were newly diagnosed as having gastric cancer at one of nine hospitals in the Tokyo metropolitan area between 1993 and 1995. Patients who had undergone treatment for gastric cancer were excluded. The diagnosis of gastric cancer was confirmed by biopsy and/or surgically resected samples. Control subjects were apparently healthy people who underwent medical checkups during the same period of recruitment. The cases and the controls were asked to provide sera, and written informed consent was obtained from all subjects.⁹ From the subjects, we randomly selected 275 cases considering sex and age. Between case and control subjects, sex and age were matched at a ratio of 1:1. From the control subjects with the same sex and age (within 2 years), one whose date of blood sample collection was the nearest to that of each case subject was selected. After the 275 cases and the 275 controls were selected, serum *H. pylori* IgG antibody was measured by an enzyme-linked immunosorbent assay using a commercial kit (J-HM-CAP; Kyowa Medex, Japan). The assay was performed according to the manufacturer's instructions in 2003. During the measurement, serum samples were analyzed in random order, and all assays were performed by a researcher who was blinded to each case/control status.

The manufacturer recommended the cutoff value of 2.30 ELISA value (EV) for current *H. pylori* infection. ORs were calculated for 5 cutoff values of 2.30, 2.00, 1.75, 1.50, and 1.25 EV. For each cutoff value, two conditional logistic regression analyses were performed to evaluate the influence of *H. pylori* infection on gastric carcinogenesis: matched for sex and age (simple model) and adjusted for smoking and drinking doses in addition to the matching variables (multivariable adjusted model). Smoking dose was classified by cigarette-year (consumed cigarettes a day multiplied by years of smoking) into five categories: 0, 1–399, 400–799, 800, or more and unknown. Drinking dose was classified by alcohol-year (pure alcohol intake in mL/day multiplied by years of drinking) into five categories: 0 (never-drinker), occasional and 0.1–134.9, 135.0–1349.9, 1350.0 or more and unknown. Subjects with missing data and whose cigarette-year or alcohol-year was not calculable were labeled unknown and kept in the analyses.

Table 1 Background of the subjects

Category	Case	Control
Male/female	142/133	142/133
Age (years)		
0–29	4 (1.5%)	4 (1.5%)
30–39	36 (13.1%)	28 (10.2%)
40–49	47 (17.1%)	58 (21.1%)
50–59	94 (34.2%)	91 (33.1%)
60–69	94 (34.2%)	94 (34.2%)
Smoking habit		$P = 0.089^\dagger$
Never	127 (46.2%)	141 (51.3%)
1–399 [‡]	39 (14.2%)	44 (16.0%)
400–799 [‡]	52 (18.9%)	42 (15.3%)
800 and more [‡]	51 (18.5%)	35 (12.7%)
Unknown	6 (2.2%)	13 (4.7%)
Alcohol intake		$P = 0.004^\dagger$
Never	92 (33.5%)	74 (26.9%)
Occasional or 0.1–134.9 [§]	46 (16.7%)	63 (22.9%)
135.0–1349.9 [§]	51 (18.5%)	57 (20.7%)
1350.0 and more [§]	68 (24.7%)	45 (16.4%)
Unknown	18 (6.5%)	36 (13.1%)

[†]Test for goodness of fit.

[‡]Cigarettes/day multiplied by years of smoking.

[§]Pure alcohol intake mL/day multiplied by years of drinking.

Table 2 Odds ratio of gastric cancer risk between *helicobacter pylori* seropositive and seronegative subjects

Kit	Seropositive (%)			
	Gastric cancer patients	Controls	Age and gender matched	Multi-variable adjusted [†]
JHM-Cap (ELISA value)				
1.25	274 (99.6%)	221 (80.4%)	54.0 (7.47, 390) [‡]	67.7 (9.14, 502) [‡]
1.50	270 (98.2%)	209 (76.0%)	31.5 (7.71, 129)	37.2 (8.82, 157)
1.75	267 (97.1%)	196 (71.3%)	18.8 (6.86, 51.3)	21.3 (7.50, 60.2)
2.00	266 (96.7%)	186 (67.6%)	21.0 (7.70, 57.3)	25.5 (8.95, 72.7)
2.30	264 (96.0%)	179 (65.1%)	22.2 (8.17, 60.6)	25.9 (9.18, 73.2)
IgG-Gap (negative: 1 and 2, and positive: 3, 4 and 5) [§]				
2.5	200 (90.9%)	180 (65.5%)	4.68 (2.85, 7.69)	5.29 (3.11, 9.00)

[†]Matched for age and gender and adjusted for smoking and alcohol intake doses with categorization shown in Table 1.

[‡]Odds ratio (95% confidence interval).

[§]The results of the previous study⁹ were used.

Subjects were 275 matched pairs, and calculation was performed using conditional logistic regression model.

To confirm that 275 selected pairs represented 788 gastric cancer patients and 1007 controls in the previous study, similar calculations were performed using previous results of *H. pylori* antibody titer measured with the “IgG-Gap” kit.⁹ Owing to the manufacturer’s recommendation, (–) and (±) were defined as negative and (+), (2+), and (3+) as positive.

Calculations were performed using EZR under R (commander Version 1.11).^{27,28}

The reanalysis of the data for the current study was approved by the Ethical Committee of Aichi Medical University School of Medicine (Issue Number: 2019-158).

Results

The backgrounds of the subjects are shown in Table 1. In comparisons of cases and controls excluding unknown results, smoking dose was higher in cases ($P = 0.02$), while no significant difference was observed in drinking dose ($P = 0.08$). Differences in the distributions of categorized data were significant only for alcohol intake. The results of the evaluation of the influence of *H. pylori* infection are shown in Table 2. For cutoff values 2.30, 2.00, 1.75, 1.50, and 1.25 EV, ORs, and their 95% confidence intervals (95% CIs) were 22.2 (8.17, 60.6), 21.0 (7.70, 57.3), 18.8 (6.86, 51.3), 31.5 (7.71, 129), and 54.0 (7.49, 39.0), respectively, in analyses matched for age and sex, and they were 25.9 (9.18, 73.2), 25.5 (8.95–72.7), 21.3 (7.50–60.2), 37.2 (8.82, 157), and 67.7 (9.14, 502), respectively, in multivariable adjusted analyses. The analysis using the results of the “IgG-Gap” kit gave an OR of approximately 5.0, which was smaller than that obtained using the “JHM-Cap” kit.

Discussion

In the current study, *H. pylori* antibody was measured in the sera of 275 gastric cancer cases and 275 controls, using a kit with a

domestic antigen. ORs and their 95% CIs were calculated for the five cutoff values equal to or less than the manufacturer’s recommendation. The point estimates were distributed between 21.3 and 67.7, around which the RR of gastric cancer risk between those with and without history of *H. pylori* infection may exist. As ORs were calculated for only five cutoff values, ORs for other cutoff values between the selected five ones might be outside of the range of 21.3–67.7. To confirm the possibility, ORs for cutoff values with 0.05 EV interval were also calculated, where no outliers were observed (Fig. 1). The ORs were larger than the OR of 5.3 by a kit with a foreign *H. pylori* antigen. One of the reasons for the larger ORs may be that the “JHM-cap” detected antibody against the CagA protein well, and the antibody is known to maintain a high titer for a relatively long time.²⁶

In Europe and North America, cohort studies evaluated the RR of gastric cancer between subjects with and without *H. pylori* infection and reported RRs of 2.8–3.6.^{5–7} In Japan, several cohort studies gave RRs of 1.8–15.^{10,29,30} Among the studies evaluating the magnitudes of *H. pylori* effect on gastric carcinogenesis, three studies have given interesting results. A Swedish case–control study showed an OR of over 20 when comparing subjects with a history of *H. pylori* infection against those without a history of *H. pylori* infection.³¹ In the study, Western blotting was used to detect low titer of *H. pylori* antibody, which gave the similar effect as lowering the cutoff value of serum antibody. The RR shown in this study seem to be compatible with that in the current study.

A Japanese cohort study, in which *H. pylori* infection was defined by results of histology, rapid urease test, and serology, showed RRs of more than 15,¹⁰ but it is difficult to infer the RR because no incidence was observed in *H. pylori*-negative subjects. In the study, at most 4% of them were thought to have had experienced loss of infection because 96% of *H. pylori*-negative patients showed none or mild atrophy. Thus, the study gave

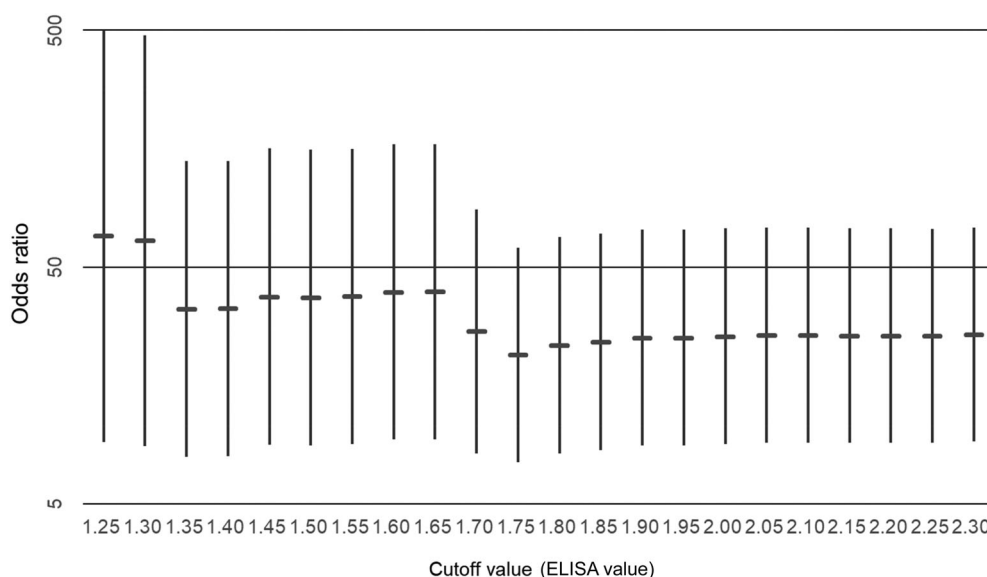


Figure 1 Odds ratio for each cutoff value of *helicobacter pylori* antibody. Point estimate (horizontal line) and 95% confidence interval (vertical line) of odds ratio for each cutoff value are shown. Vertical axis (odds ratio) is logarithmic.

relatively high RR, which seems to be consistent with the relatively high RR of the current study.

Another Japanese serological cohort study³⁰ measured both serum *H. pylori* antibody and pepsinogen values. In the study at baseline, 213 (1.1%) of the 19 106 subjects showed low titer of *H. pylori* antibody and advanced serological (indicated by serum pepsinogen values) atrophy. Since those without *H. pylori* infection are at most 10% of patients with gastric mucosal atrophy in Japan,³² most of the 213 subjects may have experienced spontaneous disappearance of *H. pylori*, and serum antibody titers may have decreased to the low level at baseline. In the cohort study, the RR in those with advanced serological atrophy irrespective of *H. pylori* antibody titer was approximately 17, when the risk of those with low antibody titer and little serological atrophy was defined as 1.0.

Another case–control study of ours, which included subjects less than 40 years of age, showed an OR of approximately 13,⁸ but the results may be underestimated because the IgG-Gap kit was used. When the difference in ORs observed in the current study depending on the used antibody kits is considered, the results of our previous study seem to be consistent with the results of the current study.

Although the sample size of the current study is too small to reach conclusive results, the results of the current study along with the several studies to date indicate that RR of gastric cancer between subjects with and without *H. pylori* infection history is more than 20 in Japan.

There are several limitations in the current study. First, the percentages of younger and female subjects were larger than those observed for gastric cancer incidence in Japan.² However, the effect of *H. pylori* infection does not depend on age or sex. Second, there might be bias in the selection of the 275 pairs from the original data set. Nonetheless, the OR calculated using the results of IgG-Gap was 5.3 in the current study, while it was 6.7 in the 788 cases and 1007 controls. It is unlikely that the selection provokes the overestimation of the RR. Furthermore, antibody measurement was performed after the selection of subjects. Little bias was expected in the selection of the subjects. Third, the time of serum collection was not recent (between 1993 and 1995). As the measurement was in 2003 and the storage situation of sera was good, the deterioration of serum quality is not expected. Between 1993 and the present, no change was expected in genetic factors in the Japanese population or in the characteristics of *H. pylori*. Although further studies are needed to reach conclusive results, the results of the current study are thought to be valid.

In conclusion, spontaneous disappearance of *H. pylori* infection may have provoked underestimation of the RR that reflects the carcinogenic effect of the infection. To reduce the effect of the spontaneous disappearance, comparison should be done between subjects with and without the infection history, and the RR is estimated to be more than 20 in Japan.

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