

Effect of Age on the Relationship between Gastric Cancer and *Helicobacter pylori*

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Helicobacter pylori is thought to be involved in the pathogenesis of gastric cancer, but the time point at which it produces its effects (critical time) is unknown. We measured the serum level of *H. pylori* antibody in 787 gastric cancer patients and 1007 controls aged 20 to 69. Odds ratios for different gastric cancer types and stages were determined for each 10-year age class. The overall odds ratio for gastric cancer decreased with age, being 7.0 for those aged 20–29, 14.5 for those aged 30–39, 9.1 for those aged 40–49, 3.5 for those aged 50–59, and 1.5 for those aged 60–69 (trend in odds ratios: $P < 0.01$). However, there was no such age-dependent trend for early diffuse-type cancer; the odds ratios were 12.6, 4.0, 7.2, 6.5, and 18.5 respectively ($P = 0.29$). Early cancer tended to show higher seroprevalence than advanced cancer, especially in older subjects. No significant difference in seroprevalence was observed between diffuse and intestinal cancers within each age-class. Seroreversion must have occurred in the time interval between the critical time and the diagnosis of the cancer, especially in older patients. The age-dependent relationship between *H. pylori* and gastric cancer may be due to seroreversion, which itself may be independent of age. This age-independence indicates that prolonged exposure to *H. pylori* does not increase the magnitude of its influence on gastric carcinogenesis. Possible mechanisms through which *H. pylori* exerts pathogenic effects are continuous inflammation in adulthood and/or irreversible damage to gastric mucosa in childhood or the teenage years.

Key words: Gastric cancer — *Helicobacter pylori* — Age — Early diffuse-type cancer

Many studies have shown that *Helicobacter pylori* is related to gastric cancer.^{1–3)} While cancer prevention trials focusing on the eradication of *H. pylori* have already been started, the critical timeframe (critical time), during which it produces its carcinogenic effect, has not been identified yet. Identification of this critical time is important because eradication of *H. pylori* after this time would have no preventive effect on the incidence of gastric cancer.

To begin to determine the critical time, we investigated the relationship between gastric cancer and *H. pylori*

with respect to age. Previous studies on the relationship between smoking and lung cancer have shown that the longer the exposure to smoking, the greater the magnitude of its effect on the incidence of lung cancer.^{4,5)} In a previous study on *H. pylori* and gastric cancer, we found a high odds ratio of 13.3 for those under 40 years of age,⁶⁾ and a meta-analysis showed that the relationship is stronger in the younger population.⁷⁾ However, other variables, such as geographic region and ethnicity, certainly affect the incidence of gastric cancer by age-class.^{8,9)} We sought to more clearly separate the influence of age from these other variables by taking a large sample from within a relatively well defined geographic and ethnic region, which is something that previous studies have largely failed to do.

We measured the level of *H. pylori* antibody in sera of 787 gastric cancer patients and 1007 controls, aged 20 to 69 years. All subjects were from a defined ethnic group from a single region of Japan. The relationship between *H.*

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pylori and gastric cancer (type and stage) was determined for several 10-year age-classes.

SUBJECTS AND METHODS

The case subjects were gastric cancer patients who were newly hospitalized in one of nine hospitals in the Tokyo Metropolitan Area between June 1993 and July 1995. Patients aged over 70 years and those who had undergone prior therapy for gastric cancer were excluded. The control subjects were recruited from several health check programs in a hospital in the same area between June 1993 and November 1994. The control subjects were recruited in order of admission; there were about 100 men and 100 women in each 10-year age-class.

The subjects were asked to provide sera and to fill out a questionnaire regarding their smoking and drinking habits. Informed consent was obtained from all subjects. Diagnosis was confirmed using the pathology reports for resection specimens or biopsy specimens. The pathology findings, including the type and stage of the cancer, were then recorded. The presence of *H. pylori* antibody was measured by the SRL Co., Ltd. (Tokyo) using Pilika-Plate G *Helicobacter* produced by Biomerica Co., Ltd. (Newport, CA). In the present study, (\pm) was defined as negative.

Both case and control subjects were classified into five 10-year age-classes, as follows: 20–29, 30–39, 40–49, 50–59, and 60–69. Odds ratios for the relationship between *H. pylori* and gastric cancer were calculated for each age-class. Odds ratios after adjusting for gender and smoking habit were also calculated. Smoking habit was categorized into never smoked or past/current smoker. The gastric cancer patients were classified into subgroups by histopathological type (intestinal/diffuse) and stage of cancer (early/advanced). Odds ratios were calculated with

the case subjects belonging to each subgroup and all control subjects, and the influence of age on seroprevalence and the odds ratios was determined. Proximal cancer was defined as any gastric cancer in which the main lesion was within the proximal third of the stomach. Odds ratios for the cancer were calculated for each of the five age-classes.

RESULTS

Table I shows *H. pylori* seroprevalence in all subjects for each age-class. The control subjects showed a significant increase in seroprevalence with age, from 28 to 81%. Most case subjects showed a seroprevalence of approximately 90%, although those aged 20–29 years showed a seroprevalence of 75%. There was no significant relationship observed between age and seroprevalence in the case subjects. There was no gender difference in seroprevalence in either case or control subjects. In age-classes except the youngest one, odds ratios were significantly elevated. The odds ratio for subjects aged 30–69 decreased with age, from 10.6 to 1.8 (Table II). This trend was significant ($P < 0.01$). Adjustment for gender and smoking habit did not change the results.

No significant difference in seroprevalence was observed between diffuse- and intestinal-type cancers (Table III). The odds ratios for each type decreased significantly with age, except in the youngest age-classes. Case subjects aged 20–39 showed a slight difference in seroprevalence between cancer stages, but the difference was not significant. Those aged 40–59 showed even less difference in seroprevalence between cancer stages (Table IV). Those aged 60–69 showed increased seroprevalence with early cancer compared with advanced cancer, with a Fisher's exact probability of 0.06. Thus the seroprevalence of *H. pylori* was not age-dependent in early cancer, but showed a decreasing trend with age in advanced cancer.

Table I. Age and Gender Distribution and *Helicobacter pylori* Seroprevalence

Age (Years)	Control subjects			Gastric cancer patients		
	Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)
20–29	101 (27.7) ^{a)}	100 (29.0)	201 (28.4)	1 (0.0)	3 (100.0)	4 (75.0)
30–39	104 (45.2)	98 (40.8)	202 (43.1)	16 (87.5)	20 (90.0)	36 (88.9)
40–49	101 (58.4)	98 (50.0)	199 (54.3)	101 (90.1)	55 (94.5)	156 (91.3)
50–59	105 (70.5)	98 (72.4)	203 (71.4)	179 (91.6)	83 (88.0)	262 (90.5)
60–69	106 (86.8)	72 (75.0)	202 (81.2)	223 (88.8)	78 (87.2)	329 (88.4)
			$P < 0.01$ ^{b)}			$P = 0.45$ ^{b)}
20–29 ^{c)}	4 (50.0)	8 (12.5)	12 (25.0)	6 (83.3)	8 (75.0)	14 (78.6)
30–39 ^{c)}	37 (43.2)	48 (31.3)	85 (36.5)	41 (87.8)	49 (91.8)	90 (90.0)

a) Number of subjects (seropositive percent).

b) P value for trend in seroprevalence.

c) Results of the previous study.⁶⁾

The odds ratios for both cancer stages decreased significantly with age.

Table V shows *H. pylori* seroprevalence in patients with early diffuse-type cancer and the odds ratios for this can-

Table II. Odds Ratios for *Helicobacter pylori* in Gastric Cancer Patients by Age

Age (Years)	Odds ratio (95% CI)	Adjusted odds ratio ^{a)} (95% CI)
20–29	7.58 (0.68, infinite)	6.95 (0.70, 69.15)
30–39	10.58 (3.40, 36.70)	14.54 (4.30, 49.17)
40–49	8.85 (4.62, 17.21)	9.06 (4.80, 17.12)
50–59	3.79 (2.21, 6.54)	3.49 (2.06, 5.90)
60–69	1.77 (1.06, 2.98)	1.49 (0.89, 2.48)
	<i>P</i> <0.01 ^{b)}	<i>P</i> <0.01 ^{b)}
20–29 ^{c)}	11.00 (1.35, 114.79)	17.43 (1.99, 153.14)
30–39 ^{c)}	15.68 (6.50, 38.88)	17.89 (7.60, 42.14)

- a) Adjusted by gender and smoking habit.
- b) *P* value for trend in odds ratios.
- c) Results of the previous study.⁶⁾

cer type. The seroprevalence significantly increased with age (*P*=0.01), while the odds ratios did not decrease with age (*P*=0.29). Seroprevalence either decreased or remained unchanged in case subjects with early intestinal-type, advanced diffuse-type, and advanced intestinal-type cancers, with *P* values of 0.71, 0.04, and 0.67 respectively; however, the odds ratios significantly decreased with age, with *P* values of 0.02, less than 0.01, and 0.04, respectively.

The numbers of patients with proximal cancer (*H. pylori* seropositivity) were 5 (100%) in those aged 30–39 years, 26 (88.5%) in those aged 40–49, 53 (88.7%) in those aged 50–59, and 76 (82.9%) in those aged 60–69. The odds ratios (95% CI) for each age-class for proximal cancer were infinite (1.1, infinite), 6.5 (1.8, 28.0), 3.1 (1.2, 8.6), and 1.1 (0.5, 2.4), respectively.

DISCUSSION

While the number of subjects in the age-classes below 40 was small in the present study, the results corroborate

Table III. *Helicobacter pylori* Seroprevalence and Odds Ratios in Patients with Diffuse- and Intestinal-type Gastric Cancers

Age (Years)	Diffuse-type cancer		Intestinal-type cancer	
	Seropositivity (<i>n</i> ^{a)})	OR ^{b)} (95% CI)	Seropositivity (<i>n</i> ^{a)})	OR ^{b)} (95% CI)
20–29	100.0% (2)	infinite (0.60, infinite)	50.0% (2)	2.53 (0.00, infinite)
30–39	87.5% (24)	9.25 (2.51, 40.35)	91.7% (12)	14.54 (1.89, infinite)
40–49	90.6% (96)	8.14 (3.72, 18.40)	93.3% (60)	11.79 (3.91, 39.87)
50–59	93.5% (108)	5.77 (2.41, 14.45)	88.3% (154)	3.02 (1.64, 5.62)
60–69	89.6% (96)	1.99 (0.90, 4.50)	88.0% (233)	1.69 (0.97, 2.98)
	<i>P</i> =0.84 ^{c)}	<i>P</i> <0.01 ^{d)}	<i>P</i> =0.52 ^{c)}	<i>P</i> <0.01 ^{d)}

Odds ratios were calculated using the control subjects in Table I.

- a) Number of subjects.
- b) Odds ratio.
- c) *P* value for trend in seroprevalence.
- d) *P* value for trend in odds ratios.

Table IV. *Helicobacter pylori* Seroprevalence and Odds Ratios in Patients with Early and Advanced Gastric Cancers

Age (Years)	Early cancer		Advanced cancer	
	Seropositivity (<i>n</i> ^{a)})	OR ^{b)} (95% CI)	Seropositivity (<i>n</i> ^{a)})	OR ^{b)} (95% CI)
20–29	100.0% (2)	infinite (0.60, infinite)	50.0% (2)	2.53 (0.00, infinite)
30–39	81.3% (89)	8.53 (3.74, 20.18)	93.9% (66)	13.06 (4.34, 43.99)
50–59	91.1% (146)	4.09 (2.06, 8.24)	89.7% (116)	3.46 (1.70, 7.19)
60–69	91.7% (169)	2.56 (1.28, 5.19)	85.0% (160)	1.31 (0.72, 2.39)
	<i>P</i> =0.52 ^{c)}	<i>P</i> <0.01 ^{d)}	<i>P</i> =0.06 ^{c)}	<i>P</i> <0.01 ^{d)}

Odds ratios were calculated using the control subjects in Table I.

- a) Number of subjects.
- b) Odds ratio.
- c) *P* value for trend in seroprevalence.
- d) *P* value for trend in odds ratios.

Table V. *Helicobacter pylori* Seroprevalence and Odds Ratios in Patients with Early Diffuse-type Gastric Cancer

Age (Years)	Early diffuse-type cancer	
	Seropositivity (n ^a)	OR ^b (95% CI)
20–29	100.0% (2)	12.57 ^c (0.60, infinite)
30–39	75.0% (8)	3.97 (0.70, 29.18)
40–49	89.6% (48)	7.24 (2.61, 21.77)
50–59	94.2% (52)	6.53 (1.86, 27.39)
60–69	100.0% (39)	18.48 ^c (1.77, infinite)
	P=0.01 ^d	P=0.29 ^e

Odds ratios were calculated using the control subjects in Table I.

a) Number of subjects.

b) Odds ratio.

c) Infinite: the odds ratios shown were amended by adding 0.5 to the 4 cells of the cross table.

d) P value for trend in seroprevalence.

e) P value for trend in odds ratios.

findings from a previous study (Tables I and II) conducted in the same hospitals. An analysis combining the data from both studies had the same results. The *H. pylori* seroprevalence among those under 40 in the current study appears to be accurate.

Seroprevalence did not depend on gender in either the case or the control subjects, and adjustment for gender had little influence on the odds ratios. While drinking habit was not related to the risk of gastric cancer, smoking, strongly suspected to be a risk factor for gastric cancer, showed a positive association. However, adjustment for smoking only minimally influenced the odds ratios and so the odds ratios without adjustment are presented. We did not perform a separate analysis for proximal cancer because there was little difference in the seroprevalence of *H. pylori* between proximal and other sites of gastric cancer.

Possible change of seroprevalence during incubation

Due to the retrospective nature of the present study, it is possible that seroconversion and seroreversion occurred during the incubation period, i.e., during the time between the critical time for *H. pylori* and the diagnosis of cancer. In both case and control subjects, seroconversion due to new infection is suspected to have occurred during the incubation period. In developed countries, infection with *H. pylori* is said to occur in at most 1% of the adult population per year.^{10–12} Seroconversion would have little effect on the relative magnitude of the odds ratios among different age-classes.

Previous studies have shown that *H. pylori* can not survive in the stomach when atrophy of the gastric mucosa advances and intestinal metaplasia occurs.¹³ While seroreversion in controls subjects with advanced atrophy could occur, it is expected to be much rarer than in case subjects. Previous studies have shown that *H. pylori* causes mucosal

atrophy and intestinal metaplasia,¹⁴ and the present study did detect increased seroprevalence of *H. pylori* in gastric cancer patients. As atrophy of the gastric mucosa progresses with time, its effect appears to be greater in older subjects. Indeed, a previous study has shown that seroreversion does occur in healthy older subjects.¹¹

In gastric cancer patients, seroreversion due to a weakened immune reaction¹⁵ or due to loss of infection^{16, 17} is also expected during the incubation period. Seroreversion may occur as a result of the natural progression of gastric cancer rendering the gastric mucosa inhospitable to *H. pylori*¹⁸ or through weakening of the immune reaction, both of which may account for the observed stronger relationship between early gastric cancer and *H. pylori* in recent studies.^{19, 20}

The present study demonstrated a slight difference in seroprevalence between early and advanced cancers in patients aged 60–69, and it is suspected that the progression of the cancer caused a seroreversion. In fact, we suspect that seroreversion may account for discrepancies in the results of epidemiological studies on the relationship between *H. pylori* infection and gastric cancer. The studies reporting negative results tend to be either prospective studies with short observation periods^{21, 22} or retrospective studies²³ consisting mainly of older subjects and subjects with cancers that are more likely to be advanced.

Diffuse- and intestinal-type cancers Intestinal-type gastric cancer, which has similar structure to intestinal mucosa and is more differentiated than diffuse-type cancer, is usually accompanied by atrophy of the gastric mucosa²⁴ and is thought to develop in the mucosa. It has been suggested that *H. pylori*, which causes atrophy of gastric mucosa, has a stronger relationship to intestinal-type than diffuse-type cancer. However, as was reported in previous studies,^{6, 25} there appears to be little difference in the seroprevalence of *H. pylori* between diffuse-type and intestinal-type gastric cancers, even after adjusting for age.^{26, 27} Thus, it seems that there is in fact no difference between diffuse- and intestinal-type cancers in the strength of the relationship with *H. pylori*.

Age-independent relationship between *H. pylori* and gastric cancer

In order to control for the effect of seroreversion due to atrophy or cancer, we performed a separate analysis focused on early diffuse-type cancer. The odds ratios did not depend on age in early diffuse-type cancer, but did in all other cancers. This further suggests that the observed age-dependent odds ratios for *H. pylori* in gastric cancer are due to seroreversion in older gastric cancer patients, and that the relationship between *H. pylori* and gastric cancer is not age-dependent.

Exposure to *H. pylori* and risk of gastric cancer In developed countries such as Japan, infection with *H. pylori* mainly occurs before 20 years of age, with infection after 20 years of age being relatively rare.²⁸ Thus, older

subjects infected with *H. pylori* are likely to have been exposed to the bacillus for a long period of time. *H. pylori* prevalence in case subjects under 40 years of age was as high as that in case subjects over 40. The relationship between *H. pylori* and gastric cancer in the younger subjects was superficially strong, though it is age-independent. Therefore, it is suggested that long exposure does not increase the magnitude of its influence on gastric carcinogenesis. This relationship is markedly different from that between smoking and lung cancer.^{4,5)}

There are at least two possible explanations for this observation. Firstly, *H. pylori* may increase the risk of carcinogenesis no matter what the time or duration of the exposure. In other words, the critical time, during which *H. pylori* exerts its pathogenic effects, is while the infection continues, but the effects are not cumulative and may be important in adulthood. Two models are consistent with this explanation; *H. pylori* has an initiator effect on carcinogenesis as does a small dose of radiation and *H. pylori* reduces the threshold value of carcinogenesis as a promoter. The explanation is supported by a Japanese study in which eradication of *H. pylori* prevented subsequent development of gastric cancer after endoscopic resection.²⁹⁾ In this case, it may be the continuous inflammation provoked by *H. pylori* that increases the risk of gastric cancer. A recent experimental study using Mongolian gerbil has suggested that *H. pylori* may exert a promoter effect in gastric carcinogenesis.³⁰⁾

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The second possible explanation is that *H. pylori* damages the gastric mucosa during childhood or the teenage years in such a manner that the damage persists into adulthood. The second explanation is supported by an American study of Japanese-Americans, in which those suspected of childhood infection with *H. pylori* had a higher risk of gastric cancer.³¹⁾ In this case, continuous infection with *H. pylori* during the early years may have irreversibly harmed the gastric mucosa, and the critical time is during the early years.

Though these explanations are not mutually exclusive, further research is needed. Properly designed intervention studies should explore more thoroughly the relationship reported here and elsewhere regarding *H. pylori* and gastric cancer.

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