

Serological Immunoglobulin G Antibody Titers to *Helicobacter pylori* in Japanese Brazilian and Non-Japanese Brazilian Gastric Cancer Patients and Controls in São Paulo

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Helicobacter pylori (*H. pylori*) infection is considered a cause of gastric cancer (GC), though evidence for this association is scarce in high-risk areas. Possible case control and/or ethnic differences were investigated as to the presence of *H. pylori* and its immunoglobulin G antibody titer in the multi-ethnic city of São Paulo, where the incidence of GC is relatively high. We performed a cross-sectional comparison of antibody titers to *H. pylori* in Japanese Brazilian, and non-Japanese Brazilian GC patients and their controls. Japanese Brazilian patients were matched by age, sex and ethnicity with two controls, while non-Japanese Brazilian patients were matched as above with one control. Among Japanese Brazilians, 59 of 93 (63.4%) patients with GC and 127 of 186 (68.3%) controls were positive for *H. pylori*-specific antibody (odds ratio (OR)=0.80, 95% confidence interval (CI)=0.47–1.36), while among non-Japanese Brazilians, 171 of 228 patients with GC (75.7%) and 178 of 226 controls (78.8%) were positive (OR=0.84, 95%CI=0.54–1.30). The median serum antibody titer was lower in cases than in controls in both ethnic groups. A high titer (*H. pylori* titer ≥ 50) was associated with less likelihood of GC for both ethnic groups (for Japanese Brazilians, OR=0.39, 95%CI=0.16–0.92; for non-Japanese Brazilians, OR=0.56, 95%CI=0.31–1.02). The high titer can be regarded as a sign of the necessity of eradication, and low titer is regarded as a sign of the necessity of close screening for GC in both ethnic groups, because extended atrophy may cause spontaneous disappearance of *H. pylori* from the stomach.

Key words: *Helicobacter pylori* — Antibody titer — Gastric cancer — Ethnicity

Since the existence of *Helicobacter pylori* (*H. pylori*) in human gastric antrum was detected by Marshall and Warren¹⁾ in 1983, studies on *H. pylori*, gram-negative spiral or curved bacilli, have become familiar to virtually everyone in the field.^{2–13)} Now *H. pylori* has been established as a causative factor in chronic atrophic gastritis, peptic ulcer disease, Mucosa-Associated Lymphoid Tissue (MALT) lymphoma and gastric cancer.¹⁴⁾ The IARC (International Agency for Research on Cancer) designated *H. pylori* as group I (definite carcinogen) based on epidemiological investigation in 1994, so that the relation between *H. pylori* and gastric cancer has now drawn world-wide attention.¹⁵⁾

Although gastric cancer is estimated to be the world's second most common carcinoma, its incidence has signifi-

cantly declined worldwide in recent decades.^{16, 17)} In Japan, gastric cancer has been the leading cause of cancer mortality,¹⁸⁾ and the prevalence of *H. pylori* infection is high.¹⁹⁾ However, the incidence and mortality rate of gastric cancer have declined over the last few decades,^{18, 20)} presumably due to environmental factors.^{21, 22)} In many areas of the world outside Japan, including Russia, China, Costa Rica, Colombia and Brazil, the incidence of gastric cancer is higher than that of any other cancer.²³⁾ Japanese immigrants to the United States (Hawaii) have exhibited a sharp decline in gastric cancer, notably among first-generation Japanese-Americans.¹¹⁾ The incidence of gastric cancer among Japanese residents (born in Japan) in the city of São Paulo was approximately two times higher than among non-Japanese Brazilians, while the rate was only 15% lower than that of Japanese living in Japan.²⁴⁾

The above findings suggest that there may be ethnic differences in the presence of serological antibody to *H.*

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pylori, if *H. pylori* infection is a determinant of gastric cancer incidence. In order to investigate the ethnic and case-control differences in serological *H. pylori* immunoglobulin G (IgG) antibody, we conducted a cross-sectional seroepidemiological study of Japanese and non-Japanese Brazilian gastric cancer patients and their non-cancer controls.

MATERIALS AND METHODS

Study population All subjects have been admitted to one of the 13 collaborating hospitals in São Paulo over a period of 3 years (June 1991 to June 1994). A hundred and one first- (born in Japan and immigrated to Brazil) or second- (born in Brazil of a Japanese mother or father who had immigrated to Brazil) generation Japanese Brazilians aged 40 to 89 years old participated in our case-control study. Diagnosis of adenocarcinoma of the stomach was established by histological examination of tumor tissue obtained by gastroendoscopy or surgery. In 8 cases, the histological diagnosis was not confirmed or pre-operative blood samples were not obtained. From the remaining 93 cases we obtained blood samples by venipuncture and centrifuged them to separate sera just after blood sampling. For each of the 93 cases identified, 2 controls matched by age (within 5 years) and sex were designated to be randomly selected from the roster of inpatients and outpatients of all the same hospitals. In only one hospital, a specialized cancer unit, the controls were selected from neighboring public hospitals. However, since it was not possible to achieve the desired matching within the hospital, 80 of 186 controls were recruited voluntarily from the Japanese community in São Paulo. As the findings obtained from 106 controls excluding the 80 volunteers

were similar to those from the volunteers, the data using all 186 controls are reported here. Patients with conditions such as other malignant or chronic disease or diabetes mellitus requiring either a special diet or involving changes in lifestyle and diet were ineligible as controls. Each subject was questioned concerning demographic variables, dietary habits, personal and family medical history and a wide range of other lifestyle variables, including tobacco smoking and alcohol consumption, by a trained interviewer.

For 250 non-Japanese Brazilian gastric cancer patients, we used the same protocol at the same time as for the Japanese Brazilians aged 40 to 70 years old. In 24 cases, histological diagnoses were not confirmed or pre-operative blood samples were not obtained. We performed serologic analysis for the remaining 226 cases (163 men and 63 women), for each of whom the sex- and age- (within 5 years) matched controls were selected in the same hospitals except one cancer hospital.

The characteristics of the Japanese and non-Japanese Brazilian gastric cancer patients and controls are shown in Table I. Of the 93 Japanese Brazilians included in this analysis, 62% were male. The mean age of the Japanese Brazilians (65 years) was 6 years older than that of the non-Japanese Brazilians (59 years). Among the 186 Japanese Brazilian controls, the most common personal medical history included cardiovascular disease (34), respiratory disease (13), infectious disease (8), metabolic disease (1), other digestive tract and abdominal disease (17, of which 6 were hernias). For 226 non-Japanese Brazilian controls, the most common were cardiovascular disease (63), respiratory disease (15), infectious disease (10), and other digestive tract and abdominal disease (76, of which 55 were hernias).

Table I. Subject Characteristics of Each Ethnic Group

Variables	Japanese Brazilians		Non-Japanese Brazilians			
	Cases (%)	Controls (%)	Cases (%)	Controls (%)		
Sample size (N)	93	186	226	226		
Male	58 (62)	116 (62)	163 (72)	163 (72)		
Female	35 (38)	70 (38)	63 (28)	63 (28)		
Age distribution (%)	<u>Years</u>		<u>Years</u>			
	40-49	9 (10)	18 (10)	40-49	37 (16)	36 ^{a)} (16)
	50-59	22 (24)	43 (23)	50-59	76 (33)	84 (37)
	60-69	26 (28)	50 (27)	60-69	103 (46)	99 (44)
	70-79	25 (27)	55 (30)	70-79 ^{b)}	10 (5)	7 (3)
	80-89	11 (12)	20 (11)			
Mean age (years)	65.8	65.4	58.4	58.2		

a) One control was 39 years old.

b) All subjects were 70 years old.

The protocol was approved by the Institutional Review Board of each hospital where the subjects were recruited, and informed consent in writing was obtained from each participant.

Serological methods Before operation or treatment, a serum blood sample was obtained by venipuncture. After centrifugation, the serum samples were stored at -80°C until transported to Japan with sufficient dry ice. They were again stored at -80°C upon arrival. The stored serum samples were tested for anti-*H. pylori* antibody using an enzyme-linked immunosorbent assay (ELISA; Helico-G, Porton-Cambridge, Newmarket, UK). This assay system was based on detection of specific IgG antibodies to *H. pylori*, and had sensitivity and specificity rates of 96% and 86%,²⁵⁾ respectively. Serum anti-*H. pylori* IgG titers were determined by optical density reading in relation to a standard curve, which was obtained through a calibrator using a kit. More than 10 units per milliliter were considered a positive test; this criterion was chosen on the basis of previous serological studies.²⁶⁾ All samples were assayed blindly with respect to case-control status and ethnic group. Samples of cases and controls were always assayed in each plate at the same time.

Statistical analysis In order to test the significance of differences between gastric cancer patients and matched controls, matched analysis was used. The sign test was used to test median differences. For Japanese Brazilians (matched with two controls), the average titer from two controls was used. Conditional logistic regression analysis was used to determine the odds ratio (OR) and 95% confidence intervals (CI) for the relationship between the serum *H. pylori* antibody level and gastric cancer status. For Japanese Brazilians after excluding volunteer controls, the conditional logistic analysis was based on category matching of cases and controls into 20 strata formed for each combination of sex and 5-year age group ranging from 40 to 89 years. Smoking (ever vs. never), alcohol consumption (every day vs. less), educational background (college/university vs. less), number of siblings (5 or more vs. less) and country

of birth (Japan vs. Brazil, only for Japanese Brazilians) were also included in the model for adjusting possible confounders.

Histological-type gastric cancers were classified into two groups: differentiated (well or moderately differentiated) and undifferentiated (poorly differentiated and undifferentiated).

RESULTS

Median serum *H. pylori* IgG antibody level and interquartile range (IQR) by case-control status, sex and ethnicity are shown in Table II. There was a statistically significant ($P<0.001$) difference between the median *H. pylori* antibody levels in gastric cancer patients and controls in Japanese Brazilians and a marginally significant difference ($P=0.08$) in non-Japanese Brazilians. The median *H. pylori* antibody titer in gastric cancer patients was approximately 2–5 units lower than in controls in both ethnic groups and both sexes. Moreover, the median antibody titer of Japanese Brazilians was 3–4 units lower than that of non-Japanese Brazilians in both case and control groups. The ethnic differences were still observed when the ages of Japanese Brazilians were restricted to up to 70 years old (median level; 11.9 in cases and 14.4 in controls).

The association of *H. pylori* antibody level and gastric cancer is shown by ORs in Tables III and IV. *H. pylori* positivity (≥ 10 units/ml) was slightly lower in cases than controls in both ethnic groups (for Japanese Brazilians, OR=0.80, 95%CI=0.47–1.36; for non-Japanese Brazilians, OR=0.84, 95%CI=0.54–1.30). There was no significant difference between crude OR and adjusted OR. Of the subjects whose antibody levels were over 50 units/ml, the proportion in gastric cancer patients was lower than controls in both ethnic groups (for Japanese Brazilians, OR=0.39, 95%CI=0.16–0.92; for non-Japanese Brazilians, OR=0.56, 95%CI=0.31–1.02). In the other strata of *H. pylori* antibody titer (e.g., 10–19, 20–49 units/ml), we

Table II. Median and Interquartile Range (IQR) of Serum *H. pylori* IgG Antibody Level (unit/ml) by Status, Sex and Ethnicity

	Japanese Brazilians			Non-Japanese Brazilians		
	Cases (IQR)	Controls (IQR)	<i>P</i> value for difference ^{a)}	Cases (IQR)	Controls (IQR)	<i>P</i> value for difference ^{a)}
Persons	11.9 (8.8–21.2)	16.1 (8.6–39.2)	<0.001	16.2 (10.0–31.8)	18.9 (10.9–46.7)	0.08
Males	12.0 (8.8–19.6)	16.5 (9.9–44.3)	<0.001	16.4 (10.4–31.8)	20.3 (10.9–46.9)	0.07
Females	11.7 (8.4–25.4)	14.4 (8.0–31.2)	0.32	15.6 (9.4–33.4)	18.5 (10.4–46.7)	0.80

a) Sign test.

Table III. Distribution of the *H. pylori* Seroprevalence and Odds Ratio (OR) and 95% Confidence Interval (CI) among Japanese Brazilians

Antibody level (unit/ml)	Cases (%)	Controls (%)	OR (95%CI) ^{a)}	Adjusted OR (95%CI) ^{b)}
<10	34 (36.6)	59 (31.7)	1.00	1.00
≥10	59 (63.4)	127 (68.3)	0.80 (0.47–1.36)	0.86 (0.49–1.53)
10–19	34 (36.6)	48 (25.8)	1.23 (0.65–2.32)	1.33 (0.67–2.62)
20–49	17 (18.3)	41 (22.0)	0.74 (0.36–1.51)	0.80 (0.37–1.75)
≥50	8 (8.6)	38 (20.4)	0.39 (0.16–0.92)	0.41 (0.17–1.02)

a) Calculated by conditional logistic analysis.

b) Calculated by conditional logistic analysis adjusted for smoking, alcohol drinking, educational background, number of siblings and country of birth. Two subjects were deleted due to missing values for some variables.

Table IV. Distribution of the *H. pylori* Seroprevalence and Odds Ratio (OR) and 95% Confidence Interval (CI) among Non-Japanese Brazilians

Antibody level (unit/ml)	Cases (%)	Control (%)	OR (95%CI) ^{a)}	Adjusted OR (95%CI) ^{b)}
<10	55 (24.3)	48 (21.2)	1.00	1.00
≥10	171 (75.7)	178 (78.8)	0.84 (0.54–1.30)	0.81 (0.51–1.28)
10–19	87 (38.5)	67 (29.7)	1.14 (0.69–1.90)	1.12 (0.66–1.89)
20–49	55 (24.3)	65 (28.8)	0.74 (0.43–1.27)	0.70 (0.40–1.23)
≥50	29 (12.8)	46 (20.4)	0.56 (0.31–1.02)	0.54 (0.29–1.00)

a) Calculated by conditional logistic analysis.

b) Calculated by conditional logistic analysis adjusted for smoking, alcohol drinking, educational background and number of siblings. Seven subjects were deleted due to missing values for some variables.

could not find any statistically significant differences, though the percentage of people among cases whose *H. pylori* antibody level was 10 units or more and less than 20 units, was higher than among controls in both ethnic groups. In both ethnic groups, OR decreased as the *H. pylori* titer level increased. In terms of ethnic difference, seroprevalence of *H. pylori* among the non-Japanese Brazilians was about 10% higher than among Japanese Brazilians in cases and controls, respectively; 63.4% (Japanese Brazilians) versus 75.7% (non-Japanese Brazilians) in cases, 68.3% (Japanese Brazilians) versus 78.8% (non-Japanese Brazilians) in controls. The ethnic differences became larger when ages of Japanese Brazilians were restricted to up to 70 years old (57.4% in cases and 64.5% in controls). When the 80 volunteers were excluded from the controls, median *H. pylori* titer was 16.4 and seropositivity was 68.9 (OR=0.81, 95%CI=0.45–1.42). The values were similar with those among all controls.

Differentiated type cancer was slightly more common than undifferentiated type cancer in both ethnic cancer patients with *H. pylori* antibody titer ≥10 units/ml (65.9% (27/41) vs. 62.5% (25/40) among Japanese Brazil-

ians, 79.6% (78/98) vs. 62.5% (64/86) among non-Japanese Brazilians, respectively), although the difference was not statistically significant by the χ^2 test. However, there was no differentiated type gastric cancer patient with high titer for *H. pylori* (≥50 units/ml) among Japanese Brazilians, while there were 7 patients (17.5%) in undifferentiated type ($P<0.001$ by Fisher's exact test). Among non-Japanese Brazilians there was no difference of patients with high *H. pylori* titer (≥50 units/ml) between differentiated type (13.3%) and undifferentiated type (12.8%) gastric cancer.

DISCUSSION

In this study we investigated the relationship between *H. pylori* titer and gastric cancer, and the ethnic difference in *H. pylori* titer. Previous ethnic studies on the prevalence of *H. pylori* infection have used seropositivity as a measure.^{27,28)} However, few studies have evaluated the significance of *H. pylori* titer.

The present investigation afforded two interesting results. First, the median levels of *H. pylori* titer among

cancer patients were lower than among control subjects in both ethnic groups (11.9 and 16.1 in Japanese Brazilian gastric cancer patients and controls, respectively; 16.2 and 18.9 in non-Japanese Brazilian cases and controls, respectively), although there were no statistically significant differences for non-Japanese Brazilians. Progression of chronic superficial gastritis to atrophic gastritis after long-term follow-up is well established from the epidemiological and experimental points of view.^{29, 30} *H. pylori* infection induces superficial gastritis, and initiates a process culminating in chronic atrophic gastritis after long-term follow-up.³¹ Forman and many other scientists considered that many cancer cases would have been likely to have had severe atrophic gastritis and intestinal metaplasia, conditions that favor the loss of *H. pylori* colonization, and subsequently, loss of seropositivity.^{32, 33} Extended atrophy may cause spontaneous disappearance of *H. pylori* from the stomach.³⁴ Our case population may have included many patients with severe mucosal atrophy, who are at higher risk for gastric cancer, and whose serum antibody titers are lower than those of the control population. We showed in another paper that gastric cancer cases in this study were associated with a pepsinogen I level <30 ng/ml and a pepsinogen ratio <3.0,³³ both being markers of atrophic gastritis.

Some previous case-control studies may possibly have misinterpreted the meaning of low titer or negative serology in cross-sectional comparison.¹⁵ The *H. pylori* titer, which may well have been high several years earlier when the cancer was diagnosed, had presumably decreased by the time of our study. To investigate the association between *H. pylori* titer and gastric cancer more accurately, one must make an assessment using blood samples provided 10 years or more before diagnosis. It is obvious that an adult with low titer should not have eradication therapy, but rather a cancer-screening check, and that only adults with high titer should undergo eradication therapy, if necessary.

Secondly, the *H. pylori* seropositivity among Japanese Brazilian controls (68.3%) was not higher than among non-Japanese Brazilian controls (78.8%). These ethnic differences were still observed after considering age distribution. However, the incidence rate of gastric cancer was not parallel to the *H. pylori* seropositivity in this study. The age-adjusted incidence rate (AAIR) (adjusted for the world population) of gastric cancer among first-generation male and female Japanese residents in the city of São Paulo is one of the highest in the world: 69.3 for males and 32.0 among females in 1969–78.²⁴ These rates were one-and-a-half times as high as those among the general Brazilian population in the city: 45.7 for males and 19.0 for females

in 1973.²⁴ However, more persons were infected with *H. pylori* among non-Japanese Brazilians. This at first appears contradictory. The *H. pylori* seropositivity among randomly selected Japanese residents aged 40 to 59 years was similar in São Paulo and in Lima, although prevalence of chronic atrophic gastritis among them was two times higher in São Paulo than in Lima in our previous study.³⁵ We believe many factors other than *H. pylori* infection, such as diet and other environmental factors, may explain this difference in the pace of gastric mucosal atrophy. Recently, it was reported that excessive NaCl intake enhanced *H. pylori* colonization in mice and humans, and that chronic salt intake may exacerbate gastritis by increasing *H. pylori* colonization.^{36, 37}

In conclusion, serological IgG antibody titer to *H. pylori* in gastric cancer patients was lower than in their controls, and the *H. pylori* seroprevalence among non-Japanese Brazilians was higher than among Japanese Brazilians, despite the lower incidence of gastric cancer. We cannot explain the correlation between *H. pylori* infection and patients' status in this study, but it appears that, besides *H. pylori*, there were a number of other factors promoting the progression to gastric cancer.

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