RESEARCH COMMUNICATION

Smoking Behavior and Risk of *Helicobacter Pylori* Infection, Gastric Atrophy and Gastric Cancer in Japanese

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Abstract

Although many studies have shown that smoking is an established risk factor for gastric cancer, relatively few studies have investigated on which step smoking has effects in *Helicobacter pylori* (*H. pylori*) related gastric carcinogenesis. In this study we investigated the association of smoking with risk of three steps leading to gastric cancer: *H. pylori* infection, gastric atrophy, and gastric cancer. Among the participants who visited Aichi Cancer Center Hospital from year 2001 to 2005, 583 cases diagnosed as gastric cancer and age-and sex-frequency-matched 1,742 cancer free controls were sampled, from whom those without serum samples or without information about smoking habit were excluded, leaving 576 cases and 1,599 controls eligible for the analyses. Anti- *H. pylori* IgG antibody and serum pepsinogens (PG) were measured to detect *H. pylori* infection and gastric atrophy. Smoking status was asked by a self-administered questionnaire. The odds ratio (OR) of *H. pylori* infection, as well as the OR of gastric atrophy among the *H. pylori* seropositive controls was not significant for smokers. The age- and sex-adjusted OR of gastric cancer was significantly elevated relative to the subjects with gastric atrophy: OR=1.62 (95% confidence interval (CI), 1.19-2.22; P=0.002) for ever smokers and 2.52 (1.75-3.64; P<0.001) for current smokers, relative to never smokers. This study revealed that smoking behavior contributed to the increased risk of gastric carcinogenesis from gastric atrophy, but had little influence on *H. pylori* infection or gastric atrophy development.

Keywords: Gastric cancer - gastric atrophy - *H. pylori* infection - smoking - lifestyle factors

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Introduction

Gastric cancer is the second largest cause of cancer death in the world (Parkin et al., 1999), and is the biggest cause in the Eastern Asian countries. Although considerable number of studies have shown that *Helicobacter pylori* (*H. pylori*) infection is an established risk factor for gastric cancer (IARC, 1994), relatively few studies have investigated the influences of lifestyle factors like smoking, alcohol consumption or nutrient intakes on gastric carcinogenesis in relation to *H. pylori* infection (Ito et al., 2003; Kato et al., 2004). Accumulated evidence indicates that there are three steps in gastric carcinogenesis: *H. pylori* infection, gastric atrophy development and carcinogenesis (Hamajima et al., 2006). While previous studies have investigated the genetic factors involved in each of these three steps (Hishida et al., 2009a; 2009b), there exist few reports that examined the roles of lifestyle factors in each step of gastric carcinogenesis. Among the several lifestyle factors smoking is shown to be a definite risk factor for gastric carcinogenesis. Recent study in Japan demonstrated that those who have both of smoking habit and *H. pylori* infection are at 11-fold higher risk of gastric cancer (Shikata et al., 2008). In this study we investigated the association of smoking with *H. pylori* infection, risk of gastric atrophy measured with serum pepsinogen levels and risk of gastric cancer using the gastric cancer cases and control subjects who visited the outpatient clinic of Aichi Cancer Center Hospital during 2001-2005 to clarify the influence of smoking on each step of gastric carcinogenesis.

Materials and Methods

Study participants

Subjects were participants of HERPACC (Hospital-based Epidemiologic Research Program at Aichi Cancer Center) study, in which first-visit outpatients were consecutively invited to provide lifestyle data and blood sample after informed consent (Tajima et al., 2000). Among the participants who visited Aichi Cancer Center Hospital from year 2001 to 2005, 583 cases diagnosed as gastric cancer and age- and sex-frequency-matched 1,742 cancer free outpatients (controls) were sampled, from whom 104 controls were excluded because of lack of serum, and 7 cases and 39 controls were excluded because the

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information about smoking habit was insufficient, leaving 576 cases and 1,599 controls eligible for the analyses. All the subjects gave written informed consent to participate in the study, completed a self-administered questionnaire and provided blood. The questionnaire included items on smoking status and alcohol consumption. Smoking status was classified as current, former or never, and level of exposure was evaluated in pack years. Former smokers were defined as people who had quitted smoking for at least 1 year. Current smokers and former smokers were combined as ever smokers in the analyses. This study protocol was approved by the Ethics Committees of Aichi Cancer Center and Nagoya University Graduate School of Medicine.

Samples and diagnostic criteria

The serum samples provided were immediately stored at -20°C until analysis. Anti-*H. pylori* IgG antibody was measured with a direct ELISA kit "E plate 'Eiken' H. pylori Antibody" (Eiken Kagaku, Tokyo, Japan). According to the instruction of this kit, 10.0 units or higher was regarded as seropositive. Serum pepsinogens (PG) were measured by chemiluminescence enzyme immunoassay (CLEIA). Gastric mucosal atrophy was grouped into "none" (PG I >70ng/ml or PG I/PG II >3), "mild" (PG I <70ng/ml and PG I/PG II <3, excluding "severe" cases), or "severe" (PG I <0ng/ml and PG I /PG II <2). Since serum samples of gastric cancer cases were planned to be used for a study with higher priority, the antibody and PGs of the cases were not measured.

Statistical analysis

The differences in proportions were examined with a Fisher's exact test. The 95% confidence intervals

Table 1. Characteristics of the Study Subjects

	Controls n=1599			Cases n=576
	H. pylori (-)	H. pylori (+)		
		GA (-)	GA (+)	
n	682	434	483	576
Sex				
Male	465 (68.2%)	356 (82.0%)	351 (72.7%)	422 (73.3%)
Female	217 (31.8%)	78 (18.0%)	132 (27.3%)	154 (26.7%)
Age				
<30	6 (0.9%)	1 (0.2%)	1 (0.2%)	2 (0.4%)
30-39	66 (9.7%)	11 (2.5%)	3 (0.6%)	31 (5.4%)
40-49	138 (20.2%)	54 (12.4%)	31 (6.4%)	64 (11.1%)
50-59	191 (28.1%)	151 (34.8%)	139 (28.8%)	211 (36.6%)
60-69	202 (29.6%)	150 (34.6%)	214 (44.3%)	164 (28.5%)
70-	79 (11.6%)	67 (15.4%)	95 (19.7%)	104 (18.1%)
Smoking Status				
Never	309 (45.3%)	139 (32.0%)	201 (41.6%)	190 (33.0%)
Former	226 (33.1%)	180 (41.5%)	191 (39.5%)	179 (31.1%)
pack year < 50	182 (80.5%)	136 (75.6%)	140 (73.3%)	134 (74.9%)
pack year ≥ 50	44 (19.5%)	44 (24.4%)	51 (26.7%)	45 (25.1%)
Current	147 (21.6%)	115 (26.5%)	91 (18.8%)	207 (35.9%)
pack year < 50	116 (78.9%)	77 (67.0%)	65 (71.4%)	132 (63.8%)
pack year ≥ 50	31 (21.1%)	38 (33.0%)	26 (28.6%)	75 (36.2%)
Ever	373 (54.7%)	295 (68.0%)	282 (58.4%)	386 (67.0%)
pack year < 50	298 (79.9%)	213 (72.2%)	205 (72.7%)	266 (68.9%)
pack year ≥ 50	75 (20.1%)	82 (27.8%)	77 (27.3%)	120 (31.1%)

GA(-), subjects with no atrophy; GA(+), subjects with gastric atrophy (PG I ≤ 70ng/ml and PG I / PG II ≤ 3).

Table 2. Smoking Status and Age- and Sex- Adjusted Odds Ratio (OR) and 95%

Smoking status[a]	n	H. pylori +	H. pylori +(%)	OR[b]	95% CI	P value
Never	649	340	52.4	1	Reference	-
Ever	950	577	60.7	1.24	0.97-1.58	0.087
Current	353	206	58.4	1.25	0.90-1.73	0.179

Confidence interval (95%CI) of H. pylori seropositivity [a] Ever smoker=Former smoker+Current smoker; [b]Adjusted for age and sex

Table 3. Distribution of Smoking Status According to *H. pylori* Seropositivity and the Grade of Gastric Atrophy

Smoking status	*H. pylori* seronegative			*H. pylori* seropositive		
	GA (-)	GA (+)	GA (++)	GA (-)	GA (+)	GA (++)
Never	295 (46.2%)	2 (11.8%)	12 (44.4%)	139 (32.0%)	155 (48.6%)	46 (28.0%)
Ever	343 (53.8%)	15 (88.2%)	15 (55.6%)	295 (68.0%)	164 (51.4%)	118 (72.0%)
Current	139 (21.8%)	5 (29.4%)	3 (11.1%)	115 (26.5%)	53 (16.6%)	38 (23.2%)
Total	638 (100%)	17 (100%)	27 (100%)	434 (100%)	319 (100%)	164 (100%)

GA(-), subjects with no atrophy; GA(+), subjects with gastric atrophy (PG I ≤ 70ng/ml and PG I / PG II ≤ 3) ; GA(++), subjects with gastric atrophy (PG I ≤ 30ng/ml and PG I / PG II ≤ 2)

Table 4. Smoking Status and Age- and Sex- Adjusted Odds Ratio (OR) and 95% Confidence Intervals (95%CIs) of Gastric Atrophy in the *H. pylori* Seropositive Subjects

Smoking status[a]	n	All gastric atrophy (%)	OR[b]	95% CI	P value	Severe gastric atrophy (%)	OR[b]	95% CI	P value
Never	340	201 (59.1)	1	Reference	-	46 (13.5)	1	Reference	-
Ever	577	282 (48.9)	0.81	0.59-1.12	0.207	118 (20.5)	1.30	0.84-2.02	0.241
Current	206	91 (44.2)	0.70	0.47-1.03	0.072	38 (18.4)	1.37	0.81-2.34	0.243

[a] Ever smoker = Former smoker + Current smoker; [b] Adjusted for age and sex.

(CIs) for percentages were calculated based on binomial distributions. Logistic regression analysis was performed for estimating odds ratios (ORs) and 95% CIs. Age was adjusted as a continuous variable in the logistic model. *H. pylori* infected subjects was defined as those with *H. pylori* seropositive or with gastric atrophy, because in the great majority of cases gastric atrophy develops after *H. pylori* infections. The OR for those with each category of smoking status was calculated relative to never smokers defined as a reference. The trends for gastric cancer development by the categories of pack-years of smoking were compared using the χ^2 test for trend. The calculations were done using the STATA version 10 (Stata Corp, College Station, TX).

Results

The characteristics of the subjects are summarized in Table 1. The mean age±standard deviation was 58.5±10.6 years (range: 25-84 years) for controls and 58.7±10.6 years (range: 27-80 years) for cases. Females were 26.7% in the controls and 26.7% in the cases. About three quarters of the controls were infected with *H. pylori*, and about one third of the controls had gastric atrophy. We tested the trend for *H. pylori* infection, gastric atrophy or gastric cancer development by sex or age categories, which revealed significant trend for higher *H. pylori* infection rate in males (P value for trend <0.001) and higher age categories (P<0.001), and for higher prevalence of gastric atrophy in higher age categories (P <0.001).

There was no significant association between smoking behavior and the *H. pylori* seropositivity, although the OR was 1.24 for ever smokers and 1.25 for current smokers compared with nonsmokers (Table 2).

There were 917 *H. pylori* seropositive subjects, among whom 483 (52.7%) subjects had atrophy. On the one hand, there were 44 (6.5%) subjects with atrophy among 682 seronegative subjects. The difference in the prevalence was statistically significant (P <0.001). Table 3 shows the distributions of *H. pylori* seropositivity and gastric atrophy according to the smoking status. Ever smoking and current smoking was significantly associated with gastric atrophy among the *H. pylori* seronegative subjects by a 2×3 Fisher's exact test (P= 0.004 and 0.021, respectively). The risk estimation of gastric atrophy according to smoking behavior revealed the age- and sex-adjusted OR of gastric atrophy among *H. pylori* seropositive subjects was 0.81 (95% CI, 0.59-1.12; P=0.207) for ever smokers and 0.70 (0.47-1.03; P=0.072) for current smokers, compared with never smokers. The age- and sex-adjusted OR of severe gastric atrophy among *H. pylori* seropositive subjects was 1.30 (0.84-2.02; P=0.241) for ever smokers and 1.37 (0.81-2.34; P=0.243) for current smokers when never smokers were defined as a reference (Table 4).

To investigate how smoking behavior contributes to the gastric carcinogenesis from gastric atrophy, we also calculated the OR of gastric cancer compared with gastric atrophy controls. The risk of gastric cancer was significantly increased for smokers, with the age- and sex-adjusted OR of gastric cancer was 1.62 (1.19-2.22; P=0.002) for ever smokers and 2.52 (1.75-3.64; P <0.001) for current smokers, relative to never smokers. When we stratified the ever smokers and current smokers by pack-years of smoking (pack years <50 and >50), the OR was 1.51 (1.09-2.08; P=0.012) for ever smokers with pack years <50, and 2.25 (1.47-3.45; P <0.001) for those with pack years >50, while the corresponding ORs for the current smokers were 2.25 (1.52-3.34; P <0.001) and 3.74 (2.18-6.41; P <0.001), respectively. There was a trend of increased risk of gastric cancer with higher category of pack-years of smoking both among the ever smokers and the current smokers (P for trend: P=0.003 and P<0.001, respectively) (Table 5).

Discussion

In this study, we found that smoking did not have significant influence on H. pylori seropositivity or the risk of gastric atrophy development, and that smoking significantly increased the risk of gastric cancer among the

Table 5. Smoking Status and Age- and Sex- Adjusted Odds Ratio (OR) and 95% Confidence Intervals (95%CIs) of Gastric Cancer Relative to the Control Subjects with Gastric Atrophy

Smoking status[a]	Gastric atrophy n = 527	Gastric cancer n = 576	OR[b]	95% CI	P value
Never	215 (40.8%)	190 (33.0%)	1	Reference	-
Ever	312 (59.2%)	386 (67.0%)	1.62	1.19-2.22	0.002
pack year < 50	230 (43.6%)	266 (46.2%)	1.51	1.09-2.08	0.012
pack year ≥ 50	82 (15.6%)	120 (20.8%)	2.25	1.47-3.45	<0.001
Current	99 (18.8%)	207 (35.9%)	2.52	1.75-3.64	<0.001
pack year < 50	71 (13.5%)	132 (22.9%)	2.25	1.52-3.34	<0.001
pack year ≥ 50	28 (5.3%)	75 (13.0%)	3.74	2.18-6.41	<0.001

[a] Ever smoker = Former smoker + Current smoker; [b] Adjusted for age and sex.

subjects with gastric atrophy, with higher OR in current smokers than in ever smokers. Although statistically insignificant, increased point estimates in OR of *H. pylori* infection for smokers were demonstrated in this study, which was in the same trends to the recent review of literature (Suzuki et al., 2006). We speculate that the biological mechanism to explain the increase in risk of *H. pylori* infection would be a reflection of decrease in *H. pylori* infection rates among never smokers, which might result from the excessive use of antibiotics in Japan and inhibitory effects of smoking against *H. pylori* eradication as discussed previously (Yoneyama and Katsumata, 2006). We also observed the increased point estimate in OR of severe gastric atrophy among smokers in this study, although statistically not significant. This is in the same trends to the previous report that revealed the impact of cigarette smoking on the development of advanced stages of gastric premalignant lesions, suggesting the detrimental effects of smoking via the production of harmful substances like nitrosamines or DNA adducts (Mirvish, 1995). These statistically insignificant hypothetical associations should be investigated further with much larger number of study subjects in the near future.

The significantly increased risk of gastric carcinogenesis observed for smokers relative to control subjects with gastric atrophy would be explained biologically as follows. Cigarette smoking is known to contain carcinogenic substances like nitrosamine or other nitrosocompounds, which are swallowed directly to smokers (Dyke et al., 1992). Greater concentrations of smoking-related DNA adducts have been detected in gastric mucosa of the smokers, while lower levels of free radical scavengers like ascorbic acids or β-carotene are also observed in smokers (Buiatti et al., 1996). Additionally, increased mRNA expression of chemokines in gastric mucosa might enhance inflammatory reactions (Shimoyama et al., 2001). In this study, we observed the higher OR of gastric cancer for current smokers compared with that for ever smokers, suggesting that the influence of smoking would have been greater if smoking behavior was continued until recently. The significant association observed between smoking status and distribution of gastric atrophy among the H. pylori seronegative subjects might be a type I error mainly attributable to the small number of those with gastric atrophy, which requires further verification with larger number of study subjects. While the present study is a hospital-based case-control study which is well-established and widely used to detect disease risk factors, further studies with different design like cohort study with large number of study subjects are also expected, which would clarify the influence of smoking behavior more directly.

The present study has several limitations. Although the *H. pylori* status of the control subjects was examined with a serology test, we did not check the CagA status. As reported in Japan, nearly 100% of *H. pylori* strains possess a functional cag pathogenicity island (cag PAI), which encodes and produces the CagA protein (Ito et al., 1997). A previous study also certified that almost all strains isolated from our Japanese subjects were East Asian cagA-positive strains (Azuma, 2004), indicating that *H. pylori* strains in our study subjects also possesses CagA. Another limitation is related to the diagnosis of gastric atrophy. This was done entirely on the basis of serum pepsinogen levels and not through histological assessment, because most of the control subjects did not undergo gastrointestinal endoscopy with biopsy. However, the pepsinogen method is well established as a surrogate marker of gastric atrophy (Miki et al., 1987; Biemond et al., 1994; Okansen et al., 2000). The validated criterion for gastric atrophy is PG I <70ng/ml and PG I/II ratio of <3.0, and that for severe gastric atrophy is PG I <30ng/ml and PG I/II ratio of <2.0, both of which are supposed to be reliable because they are widely used in practice in Japan (Inoue et al., 1998; Yanaoka et al., 2008). Concerning the gastric cancer cases the *H. pylori* seropositivity and pepsinogen levels were not examined, but most of the gastric cancer cases in Japan seemed to be *H. pylori* positive cases (more than 90%) with gastric atrophy (more than 80%) (Sasazuki et al., 2006; Suzuki et al., 2007). However, considering that intestinal type of gastric cancer, the predominant type of gastric cancer in Japan, arises from gastric atrophy caused by *H. pylori* infection, and diffuse type gastric cancer occurs regardless of gastric atrophy (Craanen et al., 1992), and it is also reported that intestinal type gastric cancer is more influenced by environmental risk factors (Sipponen et al., 1987), it would seem intriguing to perform the subgroup analysis according to these two histological types, which might unveil the association of smoking behavior with the steps of gastric carcinogenesis more clearly, although we couldn't do it because of the unavailability or insufficiency of the histological data.

In conclusion, the present study revealed that smoking behavior contributed to the increased risk of gastric carcinogenesis from gastric atrophy, and smoking had little influence on *H. pylori* infection or gastric atrophy development. Further investigations are required to confirm our findings by cohort studies with a large number of subjects.

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