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ORIGINAL ARTICLE

Interactions between *CagA* and smoking in gastric cancer

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Abstract

AIM: To examine the interactions between cytotoxin-associated gene (*CagA*) positive *Helicobacter pylori* infection and smoking in non-cardiac gastric cancer.

METHODS: A case-control study (257 cases and 514 frequency-matched controls) was conducted from September 2008 to July 2010 in Xi'an, China. Cases were newly diagnosed, histologically confirmed non-cardiac cancer. Controls were randomly selected from similar communities to the cases and were further matched by sex and age (± 5 years). A face-to-face interview was performed by the investigators for each participant. Data were obtained using a standardized questionnaire that included questions regarding known or suspected lifestyle and environmental risk factors of gastric cancer. A 5 mL sample of fasting venous blood was taken. *CagA* infection was serologically detected by enzyme-linked immunosorbent assays.

RESULTS: Smoking and *CagA* infection were statistically significant risk factors of non-cardiac cancer. *CagA* was categorized in tertiles, and the odds ratio (OR) was 12.4 (95% CI: 6.1-20.3, $P = 0.003$) for *CagA* after being adjusted for confounding factors when the high-exposure category was compared with the low-exposure category. Smokers had an OR of 5.4 compared with subjects who never smoked (95% CI: 2.3-9.0, $P = 0.002$). The OR of non-cardiac cancer was 3.5 (95% CI: 1.8-5.3) for non-smokers with *CagA* infection, 3.5 (95% CI: 1.9-5.1) for smokers without *CagA* infection, and 8.7 (95% CI: 5.1-11.9) for smokers with *CagA* infection compared with subjects without these risk factors. After adjusting for confounding factors, the corresponding ORs of non-cardiac cancer were 3.2 (95% CI: 1.5-6.8), 2.7 (95% CI: 1.3-4.9) and 19.5 (95% CI: 10.3-42.2), respectively. There was a multiplicative interaction between smoking and *CagA*, with a synergistic factor of 2.257 ($Z = 2.315$, $P = 0.021$).

CONCLUSION: These findings support a meaningful interaction between *CagA* and smoking for the risk of gastric cancer which may have implications for its early detection.

Key words: Non-cardia cancer; Cytotoxin-associated gene; *Helicobacter pylori*; Interaction; Smoking

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INTRODUCTION

Gastric cancer is a multifactorial disease whose pathogenesis is still uncertain. *Helicobacter pylori* (*H. pylori*) infection, a class I human carcinogen, has been identified as a major risk factor of non-cardiac gastric cancer[1,2], particularly cytotoxin-associated gene (*CagA*) positive *H. pylori* infection[3-5]. Recently, smoking has been recognized as an important risk factor associated with the development of gastric cancer[6]. A meta-analysis has suggested that the risk of gastric cancer increased by approximately 50% in smokers compared with non-smokers[7]. In addition, smoking was also shown to increase the risk of gastric intestinal metaplasia (a precancerous lesion) in subjects with *H. pylori* infections[8,9], suggesting that smoking may be involved in altering or modifying the effect of *H. pylori* in gastric carcinogenesis. However, there have been few studies on the potential synergistic association between *H. pylori* infection and smoking for gastric cancer risk[10-13].

The aim of this study was to examine the associations between *H. pylori* infection and smoking and the risk of non-cardiac gastric cancer.

MATERIALS AND METHODS

From September 2008 to May 2010, patients clinically diagnosed with non-cardiac gastric cancer from Grade Ⅲ Level A comprehensive hospitals (ranked among the best hospitals) in Xi'an, China, were identified. They were clinically and pathologically diagnosed with non-cardiac gastric cancer, aged 30-79 years, and living in Xi'an at the time of their diagnosis. These patients or their family members (in some cases) signed informed consent forms to participate in this study. A total of 257 cases and 514 frequency-matched controls were enrolled. For each case, two controls were randomly selected from the same residential community and matched by sex and age (± 5 years). The controls had never been diagnosed with cancer, diabetes, or gastrointestinal disorders. A face-to-face interview was performed by the investigators for each participant using a standardized questionnaire that included questions regarding a wide variety of known or suspected lifestyle and environmental risk factors of gastric cancer.

The questionnaire included the history of socio-demographic characteristics, physical activity, medical history, family history of cancer, alcohol consumption, smoking and lifestyle factors. "Never Smoked" was defined as having smoked less than 100 cigarettes in the participant's lifetime. Quantitative smoking measures included the average number of cigarettes consumed per day and the age when started smoking, and (among former smokers) years since smoking ceased. A sample of 5 mL fasting venous blood was collected from the participants and the sera were isolated and stored at -80℃ until assayed. The antibody to *H. pylori* was tested in batched serum samples using an enzyme-linked immunosorbent assays (San Diego, CA). *CagA*-positive *H. pylori* infection was defined as the presence of *CagA* antibodies in the serum.

A conditional logistic regression model was used to estimate the odds ratios (ORs), 95% CI, and the risk factors using SPSS (version 15.0). *P* values less than 0.05 (two-tailed) were considered statistically significant. To estimate the linear association between *CagA* positive *H. pylori* infection and the risk of non-cardiac gastric cancer, *CagA* was classified into three categories (tertiles), at the nearest tertile based on the distribution in the control group. Smoking status was classified into never and ever smoking. We assessed the joint effects of smoking and *CagA* infection using four categories: *CagA* (-) and never smoked, *CagA* (-) and smoking, *CagA* (+) and never smoked, and *CagA* (+) and smoking. A synergy index (SF) was calculated in terms of the adjusted ORs. The SF is defined as:

$$SF = OR_{AB} \Big/ \left(OR_{\bar{A}B} \times OR_{A\bar{B}}\right)$$

and is the ratio of the observed *OR* for both factors combined, to the predicted *OR* assuming independent effects of each factor.

SF > 1, is defined as a positive interaction between the two risk factors, and SF < 1, means a negative interaction. The opposite applies to protective factors. To obtain the statistical significance of the *SF*, a test of interaction was performed using the *Z* statistic[14].

$$Z = \frac{In\left[OR_{AB} \Big/ \left(OR_{\bar{A}B} \times OR_{A\bar{B}}\right)\right]}{\sqrt{1/n_1 + 1/n_2 + 1/n_3 + 1/n_4 + 1/n_5 + 1/n_6 + 1/n_7 + 1/n_8}}$$

In this equation, n_1, n_2...n_8 are the values of the 8 cells in the 4 × 2 crosstable. Since the null value is 0, the statistic *Z* has asymptotically a standard normal distribution under the null hypothesis of no interaction.

RESULTS

The mean ages of the patients and the controls in this study were 56.4 and 58.2 years, respectively (Table 1). The majority of the participants were male (72.7%) in both cases and controls. Ninety-three percent of the cases and 91.3% of the controls were ethnic Han. Sixty-seven percent of the cases and 72.8% of the controls had a BMI level greater than 25.

Table 2 shows the smoking habits of the cases and controls. The proportion of current smokers and former smokers was significantly higher in the cases than in the controls. Among smokers, however, cases and controls reported a similar smoking intensity (21 and 22 cigarettes per day on average) and the pack-years of consumption were 28 and 25, respectively.

CagA positive *H. pylori* infection was strongly associated with non-cardiac cancer in this study (Table 3). *CagA* was categorized in tertiles, and the OR was 12.4 (95%

Table 1 Basic characteristics of cases and controls

Demographic data	Cases (n = 257)	Control (n = 514)	χ^2	P
Race				
Chinese Han	240	469	1.061	0.303
Others	17	45		
Education level (yr)				
< 6	82	175	2.075	0.557
6-9	87	155		
9-12	47	110		
> 12	41	74		
BMI (kg/m^2)				
≤ 25	172	374	2.824	0.093
> 25	85	140		

BMI: Body nass index.

Table 2 Smoking habits of cases and controls

	Cases (n = 257)	Control (n = 514)
Non-smoker (%)	90 (35)	355 (69)
Current smoker (%)	69 (27)	56 (11)
Former smoker (%)	98 (38)	103 (20)
Smoker (%)	167 (65)	159 (31)
Age of starting smoking	19 (15-20)	18 (15-20.5)
Cigarettes/d	21 (15-27)	22 (10-30)
Pack/yr	28 (16-42)	25 (10-39)

Data represent means, with interquartile ranges in parentheses.

CI: 6.1-20.3, P =0.003) for *CagA* after being adjusted for confounding factors when the high-exposure category was compared with the low-exposure category. Smoking was associated with the risk of non-cardiac gastric cancer. Subjects who smoked had an OR of 5.4 compared with those who never smoked (95% CI: 2.3-9.0, P = 0.002).

Smoking and *CagA* positive *H. pylori* infection had a joint effect in the development of non-cardiac cancer in this study (Table 4). In the absence of *CagA*, smoking was associated with a moderate increase of the risk in non-cardiac cancer (adjusted OR: 2.7). However, the presence of *CagA* and smoking was strongly associated with the risk of non-cardiac cancer (with an adjusted OR = 19.5), suggesting a synergistic interaction between these two factors in the development of non-cardiac cancer. The test for interaction showed that there was a multiplicative interaction between smoking and *CagA* with a synergistic factor of 2.257 (Z = 2.315, P = 0.021).

DISCUSSION

In this case-control study, smoking and *CagA* positive *H. pylori* infection was found to be important risk factors in non-cardiac gastric cancer. When both of these risk factors were present, the risk of non-cardiac gastric cancer was synergistically higher. These results suggest that smoking may somehow influence the carcinogenic processes associated with *CagA* positive *H. pylori* infection. thereby increasing the risk of gastric cancer.

Several previous studies have investigated the association between *H. pylori* infection and smoking in gastric cancer[10,12,13]. Siman and colleagues showed that among *H. pylori* seropositive subjects, smoking was associated with an increased risk of gastric cancer compared with *H. pylori* positive nonsmokers[12]. Similarly, Brenner and coworkers showed that the relative risks of gastric cancer were 2.6 for non-smoking subjects with *CagA* positive *H. pylori* infections and 7.2 for smoking subjects with *CagA* positive infections compared with subjects without smoking and *H. pylori* infection[10]. These findings were statistically significant, and are consistent with those of a case-control study in Russia, which suggested that smoking was only associated with risk of gastric cancer in men with *H. pylori* infection (OR = 2.3, CI = 1.1-4.7)[13].

Overall, it seems that smoking may increase the risk of gastric cancer in individuals with *H. pylori* infection. However, only Zaridze's study in Moscow formally examined the interaction between smoking and *H. pylori* infection and the P value for interaction was not significant. This may be due to the fact that these studies analyzed smoking and *H. pylori* infection in subjects with all types of gastric cancers, thereby potentially diluting the otherwise stronger effects they may have observed among non-cardiac cancers[2].

As mentioned above, two studies explored the association between *H. pylori* infection and smoking in non-cardiac cancer[10,11]. One study found an adjusted OR for non-cardiac cancer of 1.9 (95% CI: 0.4-8.8) for smokers without *H. pylori* infection, 6.4 (95% CI: 2.1-19.7) for never smokers with *H. pylori* infection, and 19.0 (95% CI: 5.4-67.2) for smokers with *H. pylori* infection. However, no significant interaction between smoking and *H. pylori* infection was found, perhaps due to the small number of *H. pylori* negative cases[11]. In the study reported by Brenner and colleagues, the relative risk of non-cardiac cancer was 6.1 (95% CI: 1.2-5.7) in *CagA*-positive smokers compared with nonsmoking subjects without *H. pylori* infection; this relative risk increased to 16.6 (95% CI: 4.3-64.2) in *CagA*-positive smokers[10]. In this study, never and former smokers were combined in the analysis of the joint effects of smoking and *H. pylori* infection. The inclusion of former smokers may have attenuated the estimates of the joint effects due to their potentially increased risk of gastric cancer compared with never smokers. Unlike the previous studies[12,13] that examined the modification of the smoking-gastric cancer association by *H. pylori* status, we separately analyzed those with non-cardiac gastric cancer, which may explain the markedly stronger association we observed in our data. In addition, our controls were randomly selected from the communities, thereby avoiding the potential selection bias in many hospital-based studies, where the appropriateness of the control group is often questionable.

The *CagA* antibody instead of the *H. pylori* antibody was analyzed in the present study. The reasons for using the *CagA* antibody included that over 90% of Chinese *H. pylori* isolates contain the *CagA* gene[15]. Antibodies

Table 3 Odds ratios of *CagA* positive *Helicobacter pylori* infection and smoking in non-cardiac cancer *n* (%)

	Cases (*n* = 257)	Control (*n* = 514)	Crude		Adjusted	
			OR	95% CI	OR	95% CI
CagA						
Tertile 1	45 (17.5)	249 (48.5)	1.0		1.0	
Tertile 2	85 (33.0)	170 (33.0)	2.3	1.2-3.9	3.8	1.4-7.2
Tertile 3	127 (49.5)	95 (18.5)	4.1	2.7-6.3	12.4[1]	6.1-20.3
P value				0		0.003
Smoking status						
Never smoked	90 (35.0)	355 (69.0)	1.0		1.0	
Smoking	167 (65.0)	159 (31.0)	3.6[a]	2.5-5.3	5.4[2,a]	2.3-9.0

[a]$P < 0.05$. [1]Adjusted for education, alcohol consumption, smoking and family history of gastric cancer; [2]Adjusted for education, alcohol consumption, family history of gastric cancer and *Helicobacter pylori* infection. CagA: Cytotoxin-associated gene; OR: Odds ratio.

Table 4 Risk and synergy index[1] of non-cardia gastric cancer according to *CagA* positive *Helicobacter pylori* infection and smoking *n* (%)

	Cases (*n* = 257)	Control (*n* = 514)	Crude		Adjusted[2]		SF	SF (95% CI)	*Z*	*P*
			OR	95% CI	OR	95% CI				
Smoking status							2.257	1.133-4.496	2.315	0.021[a]
CagA (-) and never smoked	30 (11.7)	269 (52.4)	1.0		1.0					
CagA (-) and smoking	45 (17.5)	105 (20.4)	3.5	1.9-5.1	2.7	1.3-4.9				
CagA (+) and never smoked	60 (23.3)	85 (16.5)	3.5	1.8-5.3	3.2	1.5-6.8				
CagA (+) and smoking	122 (47.5)	55 (10.7)	8.7	5.1-11.9	19.5	10.3-42.2				

[a]$P < 0.05$. [1]Synergy index, is the ratio of the observed odds ratio (OR) for both factors combined, to the predicted OR assuming independent effects of each factor; [2]Adjusted for education, alcohol consumption and family history of gastric cancer.

against *CagA* may have persisted longer than the antibodies against other strains of *H. pylori*[16,17]. If so, the *CagA*-Ab would better represent past exposure than the *H. pylori*-Ab. Using the *H. pylori*-Ab as a biomarker of past *H. pylori* infection would underestimate the true association between smoking and *H. pylori* infection in non-cardiac gastric cancer. Nevertheless, one previous study that used *CagA* as an indicator of *H. pylori* infection showed no significant interaction between this factor and smoking[10], possibly because this study used colorectal cancer patients as controls which have been shown to associate positively with cigarette smoking[18-20].

At present, the interaction between smoking and *CagA* positive *H. pylori* is biologically plausible. For example, bile salt reflux and gastric bile salt concentrations are higher in smokers than in nonsmokers[21]. Bile reflux was positively associated with the severity of glandular atrophy, chronic inflammation and lamina propria edema. Bile reflux causes reactive gastritis and modifies the features of *H. pylori* associated with chronic gastritis. Chronic gastritis has been implicated in gastric carcinogenesis[22]. Moreover, subjects with high bile acid concentrations and *H. pylori* infection had an elevated prevalence of intestinal metaplasia, which is also associated with the development of gastric cancer[22]. In addition, the concentration of vitamin C in gastric juices is lower in smokers[23], resulting in reduced scavenging of free radicals that may in fact be enhanced by *H. pylori*, and ultimately inhibit the growth of *H. pylori*[24]. Thus, smokers may lack vitamin C in their gastric juices that likely protect against carcinogens and inhibit *H. pylori* growth[24].

A known limitation of case-control studies is their inherent susceptibility to information and selection bias. In this study, *H. pylori* infection was estimated at the time of sample collection in cases when their gastric cancer was diagnosed. However, the presence of *H. pylori* infection typically initiates much earlier, and the signs of infection might diminish with advancing premalignant lesions[17,25]. Thus, the status of *H. pylori* infection in this study may have been misclassified which could have biased our findings towards the null association. Additionally, our sample size was not optimal for the analysis of the joint effects between smoking and *H. pylori* infection which resulted in wide confidence intervals for some risk factors.

In summary, we reported a significant interaction between smoking and *CagA* positive *H. pylori* infection for the risk of non-cardiac gastric cancer. These findings may have implications for preventive measures aimed at the early detection of gastric cancer.

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COMMENTS

Background

Helicobacter pylori (*H. pylori*) infection has been identified as a major risk factor of non-cardiac gastric cancer, particularly cytotoxin-associated gene (*CagA*) positive infection. Smoking has also been recognized as an important risk fac-

tor associated with the development of gastric cancer. In addition, smoking was shown to increase the risk of gastric intestinal metaplasia (a precancerous lesion) in subjects with *H. pylori* infections, suggesting that smoking may be involved in altering or modifying the effect of *H. pylori* in gastric carcinogenesis.

Research frontiers

Recently, an increasing number of studies have investigated the interactions between risk factors and gastric cancer, because gastric cancer is a multifactorial disease. The authors investigated the interactions between the risk factors and gastric cancer, and the findings support the effect modification by CagA positive *H.pylori* infection and smoking in the risk of non-cardiac gastric cancer.

Innovations and breakthroughs

Few studies have investigated the association between *H. pylori* infection and smoking in gastric cancer, and even fewer have formally examined the interaction between smoking and *H. pylori* infection.

Applications

This paper reported a significant interaction between smoking and *CagA* positive *H.pylori* infection in non-cardiac gastric cancer risk. These findings may have implications for preventive measures aimed at the early detection of gastric cancer.

Terminology

CagA: is the major virulence factor of type I *H. pylori*. *CagA* positive *H. pylori* infection: Infection with *H. pylori*, especially with strains carrying the *CagA*.

Peer review

Increases in numbers and concentration of a particular serotype *CagA*, as well as modeling interaction with smoking, represent improvements in exposure assessment over previous studies that have examined the relationship between *H. pylori* and smoking with respect to stomach cancer risk.

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