Smoking, *Helicobacter pylori* Virulence, and Type of Intestinal Metaplasia in Portuguese Males

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Abstract

High-virulence *Helicobacter pylori* strains and smoking increase the risk of gastric precancerous lesions. However, its association with specific types of intestinal metaplasia has been poorly studied. We aimed to quantify the association between different types of intestinal metaplasia (complete, incomplete, and mixed) and these two risk factors. Male volunteers ($n = 227$) underwent an upper digestive endoscopy and completed symptoms and lifestyle questionnaires. A histologic diagnosis was assigned based on the lesions found in any of the biopsy specimens (antrum, body, or incisura). *H. pylori vacA* and *cagA* were directly genotyped by multiplex PCR and reverse hybridization. Each participant's smoking status at the time of endoscopy was assessed. Logistic and multinomial logistic regression models were fitted (including *H. pylori* virulence, smoking, age, and education as independent variables) using normal/chronic nonatrophic gastritis as the reference category. Compared with never smokers infected with low-virulence strains, the risk of intestinal metaplasia was increased in subjects infected with high-virulence strains [odds ratio (OR), 5.74; 95% confidence interval (95% CI), 1.68-19.63] and in ever smokers (OR, 3.54; 95% CI, 1.30-9.61). In ever smokers infected with high-virulence *H. pylori* strains, the risk of intestinal metaplasia was further increased (OR, 8.61; 95% CI, 3.07-24.17). Infection with high-virulence strains significantly increased the risk of incomplete (OR, 9.81; 95% CI, 2.39-40.31) and mixed (OR, 3.28; 95% CI, 1.51-7.14) intestinal metaplasia. Complete (OR, 2.82; 95% CI, 1.01-7.88) and mixed (OR, 2.97; 95% CI, 1.12-7.84) intestinal metaplasia were more frequent among ever smokers. High-virulence *H. pylori* strains and smoking are differentially associated with the complete and incomplete types of intestinal metaplasia, suggesting divergent pathways in gastric carcinogenesis. (Cancer Epidemiol Biomarkers Prev 2007;16(2):322–6)

Introduction

It is widely accepted that ''intestinal'' type gastric carcinomas are preceded by atrophic gastritis, intestinal metaplasia, and dysplasia, following a set of sequential steps (1) amenable to modulation by environmental exposures. The association between specific *Helicobacter pylori* genotypes (*cagA*$^+$, *vacA* s_1, and *vacA* m_1) and each of the steps that precede gastric carcinoma has been described (2-8). In addition, several studies addressed the association between different lifestyles and the occurrence of precancerous gastric lesions (9, 10), smoking being the most extensively studied and the one that more strongly increases the risk of intestinal metaplasia and intestinal metaplasia progression (10-18).

Histopathologic and histochemical studies allowed the identification of two main types of intestinal metaplasia: complete and incomplete. Their patterns of mucin expression favor the hypothesis of two alternative pathways for the metaplastic changes (19) rather than a single pathway based on successive steps of phenotypic modification of the gastric mucosa (1). The evaluation of specific risk factors for these end points has not been done before and may clarify the gastric carcinogenesis pathways and the role of environmental exposures in the etiology of cancer.

We therefore quantified the association between different types of intestinal metaplasia (complete, incomplete, and mixed) and infection with high-virulence *H. pylori* strains (simultaneously *cagA*$^+$ and *vacA* s_1/m_1) and smoking.

Materials and Methods

Workers from the Viana do Castelo shipyard, north of Portugal, were invited to participate in a gastric pathology survey in 1998. Four hundred sixty (435 men and 25 women), nearly 40% of all the workers, volunteered to the study and completed a physician-administered questionnaire on digestive symptoms and had a blood sample drawn. An upper digestive endoscopy was done to 354 participants presenting dyspeptic symptoms or serum immunoglobulin G antibodies to *H. pylori*.

Biopsy specimens (one from corpus, one from antrum, and two from incisura angularis) were taken from each subject. The histologic diagnosis was based on the most severe lesion, following this descending order: intestinal metaplasia, chronic atrophic gastritis, and chronic nonatrophic gastritis. According to mucin markers (19), subjects were further classified as having complete intestinal metaplasia, incomplete intestinal metaplasia, or mixed intestinal metaplasia (if features of both complete and incomplete types were observed in the same biopsy or in different regions of the stomach). No cases of cancer or dysplasia were observed.

H. pylori cagA and *vacA* (*s* and *m*) genotypes were directly determined in the gastric biopsy specimen from the greater curvature of the antrum by multiplex PCR and reverse hybridization as previously described (20). Each subject was classified as infected with high-virulence (simultaneously *cagA*$^+$ and *vacA* s_1/m_1, *vacA* s_1/m_2, or *vacA* s_2/m_1) or low-virulence strains (otherwise). Subjects simultaneously infected

Received 10/20/06; revised 11/22/06; accepted 12/6/06.

This work was performed using grants from the Fundação Calouste Gulbenkian (projects FC-54918 and FC-68697).

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doi:10.1158/1055-9965.EPI-06-0885

with *vacA* s_1 and s_2 strains or *vacA* m_1 and m_2 strains were considered as infected with high-virulence strains.

All participants were asked to fill out an additional self-administered questionnaire that included life-course information on smoking habits and alcohol consumption. Each subject was classified as never, former smoker (for ≥6 months), and current smoker (of any amount of cigarettes) at the time of the endoscopy. For current and former smokers, the lifetime consumption in pack-years was computed by multiplying the number of years that each subject has smoked by the average daily tobacco consumption expressed in number of packs (1 pack = 20 cigarettes). Additionally, smoking habits were dichotomized to never and ever, the latter including current and former smokers. For alcohol drinking (wine, beer, hard liquor, all alcoholic beverages), the participants were categorized according to the frequency of consumption at the time of the endoscopy: up to 1 drink per week, between 2 and 6, and ≥7 drinks per week.

Given the small number of women in the sample, we only present data for male participants. Only 11 subjects presented chronic atrophic gastritis as the severest lesion and were excluded from the analyses because the small number of subjects with these conditions does not allow the assessment of its specific risk factors. After these exclusions, complete information was available for 227 infected subjects, except for tobacco consumption in pack-years (n = 217) and for drinking habits (n = 213). The median age of the subjects in the final sample was 43.3 years (range, 25-59 years). Fifty-seven (25.1%) subjects had ≤4 education years, 128 (56.4%) had 5 to 9 years, and 42 (18.5%) studied for ≥10 years.

Among subjects undergoing endoscopy, there were no significant differences between those for whom we had

Table 1. Association between intestinal metaplasia and socio-demographic, *H. pylori* virulence, and lifestyle variables (logistic regression analysis)

	Normal stomach/chronic nonatrophic gastritis	Intestinal metaplasia		
	n (%)	*n* (%)	Crude OR (95% CI)	Adjusted OR (95% CI)*
Age (y)				
<40	61 (41.5)	22 (27.5)	1 [reference]	1 [reference] †
40-50	76 (51.7)	44 (55.0)	1.60 (0.87-2.96)	1.66 (0.88-3.14)
>50	10 (6.8)	14 (17.5)	3.88 (1.51-10.00)	4.01 (1.48-10.84)
Education (y)				
≤4	35 (23.8)	22 (27.5)	1 [reference]	1 [reference] ‡
5-9	81 (55.1)	47 (58.8)	0.92 (0.48-1.76)	1.24 (0.62-2.49)
≥10	31 (21.1)	11 (13.7)	0.56 (0.24-1.35)	0.83 (0.33-2.09)
cagA genotype				
Negative	79 (53.7)	17 (21.2)	1 [reference]	1 [reference]
Positive	68 (46.3)	63 (78.8)	4.30 (2.30-8.05)	4.70 (2.46-9.01)
vacA s genotype				
s_2	68 (46.2)	12 (15.0)	1 [reference]	1 [reference]
s_1	52 (35.4)	57 (71.2)	6.21 (3.02-12.76)	7.18 (3.35-15.36)
s_1/s_2	27 (18.4)	11 (13.8)	2.31 (0.91-5.86)	2.50 (0.96-6.54)
vacA m genotype				
m_2	83 (56.5)	21 (26.2)	1 [reference]	1 [reference]
m_1	34 (23.1)	49 (61.3)	5.70 (2.98-10.90)	5.93 (3.02-11.62)
m_1/m_2	30 (20.4)	10 (12.5)	1.32 (0.56-3.12)	1.16 (0.48-2.80)
Smoking status				
Never smoker	52 (35.4)	16 (20.0)	1 [reference]	1 [reference]
Former smoker	50 (34.0)	28 (35.0)	1.82 (0.88-3.76)	1.78 (0.84-3.76)
Current smoker	45 (30.6)	36 (45.0)	2.60 (1.28-5.29)	3.19 (1.51-6.73)
Tobacco use (pack-years)§				
Never smoker	52 (36.6)	16 (21.3)	1 [reference]	1 [reference]
<20	50 (35.2)	27 (36.0)	1.76 (0.84-3.64)	2.02 (0.95-4.33)
≥20	40 (28.2)	32 (42.7)	2.60 (1.25-5.38)	2.65 (1.26-5.58)
Alcohol consumption (drinks/wk)‖				
≤1¶	13 (9.3)	5 (6.8)	1 [reference]	1 [reference]
2-6	25 (18.0)	10 (13.5)	1.04 (0.29-3.69)	1.09 (0.30-3.95)
≥7	101 (72.7)	59 (79.7)	1.52 (0.52-4.47)	1.35 (0.45-4.06)
Wine consumption (drinks/wk)**				
≤1	14 (10.3)	6 (8.3)	1 [reference]	1 [reference] ††
2-6	27 (19.8)	11 (15.3)	0.95 (0.29-3.11)	0.83 (0.22-3.16)
≥7	95 (69.9)	55 (76.4)	1.35 (0.49-3.72)	1.03 (0.30-3.47)
Beer consumption (drinks/wk) ‡‡				
≤1	53 (40.2)	28 (38.9)	1 [reference]	1 [reference] ††
2-6	51 (38.6)	34 (47.2)	1.26 (0.67-2.37)	1.56 (0.75-3.25)
≥7	28 (21.2)	10 (13.9)	0.68 (0.29-1.59)	0.64 (0.23-1.78)
Hard liquor consumption (drinks/wk)§§				
≤1	79 (59.0)	48 (64.0)	1 [reference]	1 [reference] ††
2-6	50 (37.3)	22 (29.3)	0.72 (0.39-1.34)	0.63 (0.30-1.30)
≥7	5 (3.7)	5 (6.7)	1.64 (0.45-5.98)	2.66 (0.57-12.33)

*Age and education adjusted, except if otherwise specified.
† Education adjusted.
‡ Age adjusted.
§Data available for 217 subjects.
‖Data available for 213 subjects.
¶Including one never drinker and one ex-drinker.
**Data available for 208 subjects.
†† Adjusted for age, education, and consumption of other types of alcoholic beverages.
‡‡ Data available for 204 subjects.
§§Data available for 209 subjects.

Table 2. Association between intestinal metaplasia and *H. pylori* virulence and smoking status (logistic regression analysis)

	Normal stomach/chronic nonatrophic gastritis	Intestinal metaplasia	
	n (%)	*n* (%)	OR (95% CI)*
Never smokers infected with low-virulence *H. pylori* strains †	38 (25.9)	6 (7.5)	1 [reference]
Never smokers infected with high-virulence *H. pylori* strains	14 (9.5)	10 (12.5)	5.74 (1.68-19.63)
Ever smokers infected with low-virulence *H. pylori* strains	64 (43.5)	30 (37.5)	3.54 (1.30-9.61)
Ever smokers infected with high-virulence *H. pylori* strains	31 (21.1)	34 (42.5)	8.61 (3.07-24.17)

*Age and education adjusted.
† ''High-virulence'' means simultaneous infection with *cagA*$^+$ and *vacA* s_1/m_1, *vacA* s_1/m_2, or *vacA* s_2/m_1; ''low-virulence'' means otherwise. Subjects simultaneously infected with *vacA* s_1 and s_2 strains or *vacA* m_1 and m_2 strains were considered as infected with ''high-virulence'' strains.

complete information and those we had not, concerning median age (43.3 versus 44.4, $P = 0.758$), dyspeptic symptoms (34.4% versus 33.3%, $P = 0.835$), and infection with *H. pylori* determined by serology (97.4% versus 95.5%, $P = 0.327$). Participants not providing complete information were significantly less educated (41.0% versus 25.1% having ≤4 education years, $P < 0.001$).

Unconditional logistic (intestinal metaplasia defined as present or absent) and multinomial logistic regression (intestinal metaplasia defined as complete, incomplete or mixed) models were fitted using STATA, version 9.2, to compute crude and adjusted odds ratios (OR), respectively, for intestinal metaplasia and intestinal metaplasia types. Subjects with normal stomach or chronic nonatrophic gastritis were the reference category in all analyses. The existence of an interaction between *H. pylori* virulence and smoking status was assessed both in the additive and multiplicative scales, respectively, computing the interaction contrast ratio (21) and including an interaction term (smoking × *H. pylori* virulence) in the logistic regression model.

Results

Eighty (35.2%) subjects presented intestinal metaplasia, complete ($n = 30$; 13.2%), incomplete ($n = 13$; 5.7%), or mixed ($n = 37$; 16.3%). The remaining 147 (64.8%) had normal stomach or chronic nonatrophic gastritis.

One hundred thirty-one (57.7%) subjects were infected with *cagA*$^+$ strains. The infecting strains were *vacA* s_1 in 109 (48.0%) participants, *vacA* s_2 in 80 (35.2%), and multiple *vacA* *s* genotypes in 38 (16.7%) subjects. Eighty-three (36.6%) individuals were infected with *vacA* m_1, 104 (45.8%) with *vacA* m_2, and 40 (17.6%) with both *vacA* *m* genotypes.

The risk of intestinal metaplasia was significantly higher in subjects older than 50 years compared with those under 40 years [OR, 4.01; 95% confidence interval (95% CI), 1.48-10.84]. Infection with *cagA*$^+$ (OR, 4.70; 95% CI, 2.46-9.01), *vacA* s_1 (OR, 7.18; 95% CI, 3.35-15.36) or *vacA* m_1 (OR, 5.93; 95% CI, 3.02-11.62) was significantly associated with intestinal metaplasia. Both current smokers (OR, 3.19; 95% CI, 1.51-6.73) and ever smokers with a lifetime consumption of ≥20 pack-years (OR, 2.65; 95% CI, 1.26-5.58) also presented a significantly increased risk of intestinal metaplasia when compared with never smokers. No statistically significant association was observed between the occurrence of intestinal metaplasia and consumption of alcoholic beverages (Table 1).

The prevalence of infection with high-virulence *H. pylori* strains was similar among never and ever smokers (35.3% versus 40.9%, $P = 0.43$). Compared with never smokers infected with low-virulence strains, the risk of intestinal metaplasia was increased 5.7-fold in never smokers infected with high-virulence strains and 3.5-fold in ever smokers with low-virulence infection. Subjects that were simultaneously ever smokers and infected with high-virulence *H. pylori* strains had the risk of intestinal metaplasia further increased (OR, 8.61; 95% CI, 3.07-24.17; Table 2). No statistically significant interaction was observed in a multiplicative scale ($\beta_{\text{smoking} \times \textit{H. pylori} \text{ virulence}} = -0.86$; $P = 0.227$) but the interaction contrast ratio was −0.56, not excluding an additive effect.

As shown in Table 3, infection with high-virulence strains significantly increased the risk of incomplete (OR, 9.81; 95% CI, 2.39-40.31) and mixed (OR, 3.28; 95% CI, 1.51-7.14) intestinal metaplasia. Complete (OR, 2.82; 95% CI, 1.01-7.88) and mixed (OR, 2.97; 95% CI, 1.12-7.84) intestinal metaplasia were more frequent among ever smokers.

Discussion

The risk of intestinal metaplasia was higher in subjects infected with high-virulence *H. pylori* strains and among smokers, and

Table 3. Association between complete, incomplete and mixed types of intestinal metaplasia and *H. pylori* virulence and smoking status (multinomial logistic regression analysis)

	Normal stomach/chronic nonatrophic gastritis	Intestinal metaplasia					
	n (%)	Complete		Incomplete		Mixed	
		n (%)	OR (95% CI)*	*n* (%)	OR (95% CI)*	*n* (%)	OR (95% CI)*
H. pylori infecting strains †							
Low-virulence	102 (69.4)	17 (56.7)	1 [reference]	3 (23.1)	1 [reference]	16 (43.2)	1 [reference]
High-virulence	45 (30.6)	13 (43.3)	1.79 (0.79-4.08)	10 (76.9)	9.81 (2.39-40.31)	21 (56.8)	3.28 (1.51-7.14)
Smoking status							
Never smoker	52 (35.4)	5 (16.7)	1 [reference]	5 (38.5)	1 [reference]	6 (16.2)	1 [reference]
Ever smoker	95 (64.6)	25 (83.3)	2.82 (1.01-7.88)	8 (61.5)	0.86 (0.24-3.02)	31 (83.8)	2.97 (1.12-7.84)

*Age, education, *H. pylori* virulence, and smoking status adjusted.
† ''High-virulence'' mean simultaneous infection with *cagA*$^+$ and *vacA* s_1/m_1, *vacA* s_1/m_2, or *vacA* s_2/m_1; ''low-virulence'' means otherwise. Subjects simultaneously infected with *vacA* s_1 and s_2 strains or *vacA* m_1 and m_2 strains were considered as infected with ''high-virulence'' strains.

was further increased when both factors were simultaneously present. Infection with *H. pylori cagA*$^+$ and *vacA* s_1/m_1 increased the risk of incomplete and mixed intestinal metaplasia, and smoking was associated with a higher frequency of complete and mixed types of intestinal metaplasia.

Our study evaluated male shipyard workers from the northern Portuguese region where the highest incidence of gastric cancer is observed (22), and this precludes the generalization of our observation to low-risk regions. Most subjects in our sample were drinkers, similarly to what is observed in Portugal (only ~5% of the Portuguese adult males are teetotallers; ref. 23). Therefore, our results about the association between alcohol consumption and intestinal metaplasia are not directly comparable to those obtained in previous studies, which compared drinkers with nondrinkers (10, 11, 13, 24). If an interaction between alcohol consumption and *H. pylori* infection status is to be considered (25), our conclusions may apply only to populations with a large proportion of drinkers, and not as much to females, which are less likely to consume alcoholic beverages (23).

The results presented in Table 1 suggest that the infection with multiple *vacA s* strains may be associated with a lower risk of intestinal metaplasia than s_1 strains, and these differences are even more pronounced when the infection with multiple *vacA m* strains is considered. In the analysis presented in Tables 2 and 3, the infection with multiple strains was included in the group defined as ''high-virulence,'' but the point estimates remained similar in all the analyses done when the subjects infected with multiple strains were excluded (data not shown), even if the association between high-virulence strains and complete intestinal metaplasia became statistically significant, and the study conclusions are not affected by this option.

It was known from previous investigations that both smoking (12-15) and infection with high-virulence *H. pylori* strains (4-6, 8) increased the risk of intestinal metaplasia. Although our study is not powered enough to detect a subtle interaction as the one that could be anticipated for these exposures, it suggests that the risk of intestinal metaplasia is further increased, in an additive scale, when both smoking and high-virulence *H. pylori* strains are present.

Our study also adds to prior knowledge the effect of smoking and *H. pylori* virulence in specific types of intestinal metaplasia, which may contribute to clarify the gastric carcinogenesis pathways. Two interpretative hypotheses seem to be plausible to fit intestinal metaplasia in the carcinogenesis process: (*a*) the classic sequence (1), where the complete type precedes the incomplete intestinal metaplasia, and (*b*) an alternative model proposed by Reis et al. (19) in which complete and incomplete intestinal metaplasia represent ab initio divergent differentiation programs. Infection with high-virulence *H. pylori* strains increases the risk of chronic atrophic gastritis (3, 26-28) and we also observed a clear association with incomplete intestinal metaplasia, but not as strong for the complete type. If atrophy, complete intestinal metaplasia, and incomplete intestinal metaplasia occurred in sequential steps, the association between infection and complete and incomplete intestinal metaplasia would be much more similar because it is not plausible that the observed effects are concealed in these closely related steps.

Most previous investigations did not show an association between smoking and the risk of chronic atrophic gastritis (10, 14, 29-32), but it is clear that cigarette use increases the risk of intestinal metaplasia (all types combined; refs. 10-15, 17, 18). In our study, smoking was significantly associated only with the complete type of intestinal metaplasia. It is plausible that smoking contributes to the progression from chronic atrophic gastritis to complete intestinal metaplasia, but we would not expect a lack of association with the incomplete type if it followed complete intestinal metaplasia because dysplasia (10, 13, 33, 34) and gastric cancer are also associated with smoking (35-37). Therefore, we may hypothesize that smoking acts in the pathways of two different end points: complete intestinal metaplasia (contributing for the progression from chronic atrophic gastritis to complete intestinal metaplasia) and incomplete intestinal metaplasia (contributing for its progression to dysplasia and gastric cancer without influencing the occurrence of incomplete intestinal metaplasia itself).

The risk of the mixed type intestinal metaplasia is increased by both factors considered. Although the magnitude of association for smoking is similar to the one observed with the complete type of intestinal metaplasia, for infection with high-virulence strains, it is intermediate between those observed for the complete and the incomplete types. This could be seen as supportive of model (a), with mixed type intestinal metaplasia being a transition step between complete and incomplete intestinal metaplasia. However, this observation may also fit model (b), with complete and incomplete intestinal metaplasia representing alternative pathways and mixed type intestinal metaplasia being the result of the coexistence of both main types, which may occur in different stomach locations when exposure to both factors occurs simultaneously.

Given the lower frequency of incomplete intestinal metaplasia, our study supports the notion that incomplete intestinal metaplasia is a more serious lesion than complete intestinal metaplasia, regardless of the carcinogenesis model that best fits the observations.

Our results improve our knowledge of gastric carcinogenesis but should be followed by further investigations in larger data sets and including other exposures that potentially modulate the progression of precancerous lesions, such as fruit and vegetables consumption (14, 15, 38-40) and host genetic factors (28, 41-44). In addition, we defined intestinal metaplasia as complete (also designated as type I) and incomplete, although the latter encompasses both types II and III (with different patterns of mucins expression; ref. 45). When all these three subtypes are considered, an alternative model for intestinal metaplasia progression may be taken into account (19), with type II intestinal metaplasia representing the first step in the intestinal metaplasia pathway, which may evolve to type I or type III intestinal metaplasia. Our results do not exclude this hypothesis, but reclassification of the incomplete intestinal metaplasia cases would be necessary to allow a further insight on this topic.

In conclusion, our study confirms that infection with high-virulence *H. pylori* strains and smoking increase the risk of intestinal metaplasia. It also shows that these factors are differentially associated with the complete and incomplete types of intestinal metaplasia, suggesting divergent pathways in gastric carcinogenesis.

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