

Gastric cancer risk in relation to *Helicobacter pylori* infection and subtypes of intestinal metaplasia

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Summary *Helicobacter pylori* (*H. pylori*) infection and intestinal metaplasia (IM) are each associated with an increased risk of gastric cancer (GC). To explore further the influences of *H. pylori* and IM on GC, *H. pylori* and subtypes of IM were evaluated in 135 sex and age-matched case and control pairs. Odds ratios (ORs) with 95% confidence intervals of developing GC were calculated for each risk factor using multiple logistic regression analysis. ORs for *H. pylori* infection and IM were 2.43 (1.29–4.65) and 4.59 (2.58–8.16), respectively, and those for different IM subtypes gave values of 0.82 (0.28–2.36) for type I, 2.03 (0.95–4.34) for type II and 39.75 (14.34–110.2) for type III. Stratification analysis by histological subtype and stage of GC showed a particularly high OR for IM in intestinal type (12.8, 4.73–34.83) and early GC (6.40, 2.25–18.18). Our data indicate that both *H. pylori* and IM are related to GC risk. Type III IM is a more specific marker of premalignancy, with relevance, in particular, to the early and intestinal type of GC.

Keywords: gastric cancer; *Helicobacter pylori*; intestinal metaplasia; odds ratio

Gastric cancer (GC) is one of the commonest cancers in the world (Parkin et al. 1993) as well as in Taiwan (Wu et al. 1994). Despite some improvement in its treatment, the 5-year survival rate of GC remains low. Therefore, further exploration of biological features and causes of GC is needed to reduce its occurrence. Both environmental and genetic factors are crucial in the multistage model of gastric tumorigenesis (Correa, 1992). In sequential changes from superficial gastritis to dysplasia and cancer, intestinal metaplasia (IM) plays a pivotal role. This is testified by a high frequency of IM in patients with GC and in their relatives, a similar topographic distribution of IM and GC, and a high IM occurrence in endemic areas with a high risk of GC (Dobrilla et al. 1994). Although most investigators agree with Stemmermann (1994) that the risk of GC is proportional to the extent of IM and although IM has been intensively explored as a possible premalignant lesion of GC, controversy exists because of many confounding factors in previous retrospective studies (Antonioli, 1994). Because of the heterogeneous nature of IM, a recent interest has been focused on the interrelationship between GC and different subtypes of IM (Jass et al. 1979; Turani et al. 1986; Filipe et al. 1994).

In recent years, abundant epidemiological data have consistently documented a higher rate of *H. pylori* infection in GC (Forman et al. 1991; Nomura et al. 1991; Parsonnet et al. 1991). *H. pylori*-induced inflammation may facilitate gastric carcinogenesis by increasing the rate of cell replication, decreasing the concentration of ascorbic acid and inducing DNA damage via reactive oxygen species (Sobala et al. 1993; Bechi et al. 1996). It is worth noting that the prevalence of IM is also closely related to *H. pylori*

infection (Rugge et al. 1996). Furthermore, chronic gastritis due to *H. pylori* infection may progress to IM and even GC (Correa, 1992; Dobrilla et al. 1994; Stemmermann, 1994). Nevertheless, few studies are available to redefine the interactions of these two factors with respect to gastric carcinogenesis. A preliminary observation has suggested that both IM and *H. pylori* infection are important targets for prevention of GC (Caselli, 1996). We undertook this study to investigate the relations of *H. pylori* and of IM and the risk of GC.

MATERIALS AND METHODS

Patients and tissues

With their informed consent, a total of 160 patients with histologically diagnosed GC were recruited consecutively for study from January 1995 to January 1997. None of them received chemotherapy before surgery. Tumours were classified as intestinal or diffuse subtypes according to the Lauren criteria (Lauren, 1965). The extent of tumour invasion was further divided into early or advanced GC according to the criteria proposed by the Japanese Research Society for Gastric Cancer (Murakami, 1971). After excluding 25 patients with mixed or undetermined histological subtypes of GC in gastrectomy specimens, 135 patients were finally included in this study. Tumorous, adjacent and non-tumorous portions were resected from surgical specimens and stored for further examination. Controls matched one-to-one with cases on age (within 3 years), sex, ethnic group and residential area were also selected from subjects receiving endoscopic examinations that did not reveal any ulcer nor tumour in the stomach. During this procedure, five specimens were biopsied including two from the lesser curvature of the antrum, one from the incisura angularis and two from the lesser curvature of the corpus of the stomach.

Received 12 September 1997

Revised 5 January 1998

Accepted 12 January 1998

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Table 1 Frequency distribution of age, gender, ethnic group, *H. pylori* infection and intestinal metaplasia of 135 matched pairs of gastric cancer cases and controls

Variable	Cases n (%)	Control n (%)
Age (year)		
≤ 40	17 (12.6)	18 (13.3)
41–50	21 (15.6)	19 (14.1)
51–60	23 (17.0)	24 (17.8)
61–70	37 (27.4)	37 (27.4)
> 70	37 (27.4)	37 (27.4)
Gender		
Male	71 (52.6)	71 (52.6)
Female	64 (47.4)	64 (47.4)
Ethnic group		
Fukienese	109 (80.7)	109 (80.7)
Hakka	10 (7.4)	10 (7.4)
Other	16 (11.9)	16 (11.9)
<i>H. pylori</i> ^a		
Negative	49 (36.3)	60 (44.4)
Positive	86 (63.7)	75 (55.6)
Intestinal metaplasia		
Negative	45 (33.3)	86 (63.7)
Positive	90 (66.7) ^c	49 (36.3)
Type I	6 (4.4)	17 (12.6)
Type II	22 (16.3)	25 (18.5)
Type III	62 (46.0)	7 (5.2)

^aSerological diagnosis by IgG antibodies to *H. pylori*. ^b $P < 0.0001$ vs controls.

Table 2 Histological features of diffuse and intestinal types of gastric cancer

Features	Diffuse type (n = 57)	Intestinal type (n = 78)
Depth of invasion		
Early	24 (42.1) ^a	23 (29.5)
Advanced	33 (57.9)	55 (70.5)
Adjacent metaplasia		
Positive	19 (33.3) ^c	71 (91.0)
Negative	38 (66.7)	7 (9.0)

^aNumbers in parentheses are percentages. ^b $P < 0.0001$ between diffuse and intestinal type.

Serological tests for *H. pylori*

An aliquot of 5 ml of heparinized blood was collected from each study subject, and the serum was separated on the same day and stored at -70°C until further testing. The titre of serum IgG antibody against *H. pylori* was determined using an enzyme-linked immunosorbent assay as previously described (Lin et al. 1993).

Histopathological determination of intestinal metaplasia and its subtypes

Specimens from patients and controls were fixed in 10% buffered formalin, embedded in paraffin, sectioned and stained with haematoxylin and eosin (H&E). If IM was present in H&E staining, a further section was stained using the high iron diamine (HID)/alcian

Table 3 Multivariate-adjusted odds ratios of developing gastric cancer for *H. pylori* infection and intestinal metaplasia subtypes

Factors	Adjusted odds ratios	95% Confidence intervals
<i>H. pylori</i>		
Negative	1.00	Referent
Positive	2.43	1.29–4.65
Intestinal metaplasia		
Negative	1.00	Referent
Positive		
Type I	0.82	0.28–2.36
Type II	2.03	0.95–4.34
Type III	39.75	14.34–110.20
I+II+III	4.59	2.58–8.16

blue (AB) technique (Lev. 1965). The HID/AB stain differentiates acidic mucin into sialomucins (blue) and sulphomucins (brown-black). Using HID/AB and H&E staining, the metaplastic lesions were further classified into three subtypes: type I, i.e. complete IM characterized by resembling normal intestinal epithelium; type II, i.e. incomplete IM expressing sialomucins but not sulphomucins; and type III, i.e. incomplete IM expressing sulphomucins. If IM of more than one subtype was present in a given sample, the case was assigned to the least mature subtype, as proposed previously (Rugge et al. 1996).

Statistical analysis

Odds ratios (ORs), i.e. estimators of relative risk, and the corresponding 95% confidence intervals (CI) of developing GC were calculated for each risk factor using multiple logistic regression analysis (Breslow and Day, 1980). Chi-square and Fisher's exact tests were used to analyse categorical data. A P -value less than 0.05 was considered to be statistically significant.

RESULTS

The frequency distribution of age, gender, ethnic groups, *H. pylori* infection and IM of GC patients and their matched controls is shown in Table 1. The age distribution of GC patients in this study was similar to that previously reported in Taiwan (Wu et al. 1994). Most study subjects were Fukienese. The frequency of *H. pylori* infection was different between GC (63.7%) and controls (55.6%), and the ORs (2.43, 95% CI 1.29–4.65) for *H. pylori* of developing GC was significant after adjustment for IM. GC cases had a higher frequency of IM than controls (66.7% vs 36.3%, $P < 0.0001$).

The histological features of diffuse and intestinal type GC are summarized in Table 2. There were 57 patients with diffuse type GC and 78 with intestinal type GC. According to the depth of invasion, the frequency of early lesion in diffuse type GC (42.1%) was higher than that of intestinal type GC (29.5%, $P = 0.18$). Intestinal type GC had a significantly higher frequency of coexistent IM (91.0%) than diffuse type (33.3%, $P = 0.0001$).

As shown in Table 3, ORs for *H. pylori* and IM of developing GC were 2.43 (1.29–4.65) and 4.59 (2.58–8.16) respectively. A significantly increased risk of GC was noted for type III IM (OR 39.75; 95% CI 14.34–110.20). The relationship between *H. pylori* and GC after stratification by the presence or absence of IM is summarized in Table 4. Seropositivity of *H. pylori* was less frequent in type III IM (33 out of 62, 53.2%) than in type I plus

Table 4 The relationship between *Helicobacter pylori* seropositivity and gastric cancer after stratification by the presence or absence of intestinal metaplasia

	Gastric cancer		
	Total case number	<i>H. pylori</i> (+) ^a case number	Positive rate (%)
Intestinal metaplasia			
Negative	45	31	68.9
Positive	90	55	61.1
Type I	6	5	83.3
Type II	22	17	77.3
Type III	62	33	53.2 ^b

^aPositive IgG antibodies to *H. pylori*. ^bType III vs type I + type II, $P = 0.04$

Table 5 Multivariate-adjusted odds ratios of developing gastric cancer for intestinal metaplasia stratified by subtype and stage of gastric cancer

Type/Stage	Adjusted odds ratio	95% Confidence interval
Intestinal type	12.83	4.73–34.83
Diffuse type	3.14	1.19–8.27
Early stage	6.40	2.25–18.18
Advanced stage	3.99	1.98–8.07

type II IM (22 out of 28, 78.6%; $P = 0.04$). The odds ratios of developing GC for IM in different histological subtypes and stages of GC are shown in Table 5. All odds ratios were significant: a particularly high OR was noted in the intestinal type GC (OR 12.83, 95% CI 4.73–34.83).

DISCUSSION

In the multistep process of gastric carcinogenesis hypothesized by Correa (1992), microenvironmental factors such as gastric atrophy and IM have been identified. The recent discovery of *H. pylori* as the main causative agent of gastritis leads many researchers to investigate the association of *H. pylori* infection with development of GC. It has been shown that *H. pylori* can increase the prevalence of IM (Rugge et al. 1996). In addition, prospective serological studies have also reported a three- to sixfold increase of risk of GC in *H. pylori*-infected individuals (Forman et al. 1991; Nomura et al. 1991; Parsonnet et al. 1991). In the present study, an OR of 2.43 to contract GC for *H. pylori*-infected subjects was consistent with those previously published. Collectively, these findings support a widely accepted consensus that *H. pylori* is an important environmental carcinogen for GC (Goldstone et al. 1996).

However, the mechanism by which *H. pylori* induces GC remains ill-defined. *H. pylori*-associated chronic gastritis may provide a background for gastric tumorigenesis, in which the intestinal type may be promoted in the atrophic stomach and the diffuse type in the non-atrophic mucosa (Sipponen et al. 1992; Rubin, 1997). However, it seems unlikely that *H. pylori* is solely responsible (Correa, 1992). Recently, it has been shown that gastritis per se rather than its underlying causes may be a more dominant risk factor for GC (Sipponen et al. 1992). Considering the likelihood that *H. pylori*, age and IM may be reciprocal confounding factors for GC, we have used a multiple logistic regression analysis, which disclosed that both *H. pylori* and

IM were risk factors for GC. Different odds ratio in developing GC for IM (OR 4.59) or for *H. pylori* infection (OR 2.43) were noted. Our results support the aforementioned hypothesis that IM is a precancerous lesion during the onset of GC (Sipponen et al. 1992; Filipe et al. 1994). This notion is noteworthy because IM is the advanced stage of gastritis and the risk factors for developing IM resemble those for developing GC (Correa et al. 1992; Dobrilla et al. 1994). Diets deficient in fresh fruits and vegetables, combined with a high salt and nitrite intake, are common for both of these two conditions (Correa et al. 1985; Stemmerman et al. 1990; Fuchs and Mayers, 1995). Chronic gastritis due to *H. pylori* also precedes IM and cancer (Sipponen et al. 1992; Goldstone et al. 1996), and accumulating evidence has shown that geographic variation in diet may influence the development of the intestinalized gastritis associated with GC (Stemmermann et al. 1990). Therefore, IM, reflecting the duration and severity of chronic gastritis as a result of the summation of all environmental insults, is a marker more specific for the precancerous lesion of GC. Further studies are needed to ascertain the concomitant relationship between *H. pylori* and other environmental factors, e.g. dietary factors, in the pathogenesis of IM and GC.

Previous studies have noted a variable frequency of IM in GC development (Stemmermann, 1994). One pathoepidemiological study noted that the prevalence of IM was correlated more with intestinal GC than with diffuse GC (Correa et al. 1970). Consistent with this finding, our results also showed that IM was preferentially associated with intestinal GC (OR 12.83) but there was a statistically insignificant difference between the ORs for intestinal and diffuse cancers (OR 3.14). Furthermore, we noted that IM was associated with early GC, as evidenced by a higher OR (6.40) than with advanced GC (OR 3.99), but there was a statistically insignificant difference between them. This finding supports the viewpoint that IM per se is more crucial in the early carcinogenic process, as similarly proposed by Solcia et al (1996), who reported that IM is more frequently found in the gastric mucosae of early rather than advanced cancer.

Three types of IM have been identified, according to the differences in enzyme production, mucus content and presence of Paneth cells (Stemmermann et al. 1994). The different variants of IM may exhibit different risks of GC development (Filipe et al. 1994); thus, type III IM showed stronger risk of GC (OR 39.75) than type II IM (OR 2.03), while type I had a low risk (OR 0.82). The preferential association of type III IM with GC supports the notion that type III IM is most often involved in the pathological transition from dysplasia to carcinoma (Antonioli, 1994).

It is generally believed that IM develops in a background of atrophic gastritis and, in such conditions, pre-existing *H. pylori* colonization of the stomach may be compromised (Forman et al. 1994). Mathialagan et al (1994) have demonstrated that the serological test only possesses a sensitivity of 59% in detecting *H. pylori* infection for patients who have gastric atrophy. Our results in Table 4 revealing a less frequent seropositivity of *H. pylori* in GC associated with type III IM seemed to support the above hypothesis. Similar results have been reported by Masci et al (1996), who showed that the positive rate of *H. pylori* was lower in precancerous lesions, such as dysplasia. It highlighted the possibility that the sensitivity of a serological test might be hampered by the association of gastric atrophy and IM in GC and the role of *H. pylori* might be underestimated.

In conclusion, our data indicate that both *H. pylori* and IM are associated with risk of developing GC and that they might be inter-related. Furthermore, type III IM is a more specific marker for a

pre-malignant lesion, especially with respect to early and intestinal-type GC.

ABBREVIATIONS

GC, gastric cancer; IM, intestinal metaplasia; OR, odds ratio; CI, confidence interval

ACKNOWLEDGEMENTS

This work was supported by grants from the National Science Council (NSC87-2314-B002-235, NSC85-2622-B002-011) and Department of Health, Executive Yuan, Taiwan (DOH86-TD-023, DOH87-TD-1045, DOH87-HR-525).

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