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Detection of serum anti-*Helicobacter pylori* immunoglobulin G in patients with different digestive malignant tumors

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Abstract

AIM: To investigate the seroprevalence of *Helicobacter pylori* infection in patients with different digestive malignant tumors.

METHODS: Enzyme linked immunosorbent assay (ELISA) was used to detect serum anti-*Helicobacter pylori* IgG antibody in 374 patients with different digestive malignant tumors and 310 healthy subjects (normal control group).

RESULTS: The seroprevalence of *Helicobacter pylori* infection was 61.50 %(230/374) and 46.77 % (145/310), respectively, in patients with digestive tumors and normal controls ($P<0.05$). The seroprevalence was 52.38 % (33/63), 86.60 % (84/97), 83.14 % (84/101), 45.24 (19/42), 51.13 % (18/35) and 44.44 % (16/36), respectively in patients with carcinomas of esophagus, stomach, duodenum, rectum, colon and liver ($P<0.01$). In patients with intestinal and diffuse type gastric cancers, the seroprevalence was 93.75 % (60/64) and 72.73 % (24/33), respectively ($P<0.05$). In patients with gastric antral and cardiac cancers, the seroprevalence was 96.43 % (54/56) and 73.17 % (30/41), respectively ($P<0.05$). In patients with ulcerous and proliferous type duodenal cancers, the seroprevalence of *H pylori* infection was 91.04 % (61/67) and 52.27 % (23/44), respectively ($P<0.05$). In patients with duodenal bulb and descending cancers, the seroprevalence was 94.20 % (65/69) and 45.20 % (19/42), respectively ($P<0.05$).

CONCLUSION: *H pylori* infection is associated with occurrence and development of gastric and duodenal carcinomas. Furthermore, it is also associated with histological type and locations of gastric and duodenal carcinomas.

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INTRODUCTION

Helicobacter pylori is a gram-negative, spiral-shaped, microaerophilic bacterium that colonizes gastric epithelium of humans[1-4]. Clinical and epidemiological studies have shown a close association between *H pylori* and gastric cancer[5-10]. However, the relationship between *H pylori* and other digestive tumors has not been clarified. In order to investigate the relationship between *H pylori* infection and different digestive cancers, we detected serum anti-*H pylori* IgG in 374 patients with different digestive cancers and 310 healthy subjects, using an enzyme linked immunosorbent assay -(ELISA).

MATERIALS AND METHODS

Materials

Populations A total of 374 patients with different digestive cancers were involved in this study, including 63 esophagus carcinomas, 97 gastric cancers (64 of intestinal type and 33 of diffuse type, and 56 in gastric antrum and 41 in the cardia as displayed under gastroscopy), 101 duodenal carcinomas (67 of ulcerous type and 44 of proliferous type, and 65 in the bulb and 42 in the descending part of duodenum as manifested under gastroscopy), 42 rectal cancers, 35 carcinomas of colon, 36 liver cancers. There were 240 males and 134 females, aged from 23 to 71 years old. At the same time, 310 healthy subjects were recruited as a control group. There was no difference in age and gender between the two groups.

Reagents and instruments The test kit for *H pylori*-IgG was provided by Bioseed Company (USA, batch Hillbough CA 94010). The enzyme-labeling meter was SLT-Spectra-I type (Bio-rad, USA).

Methods

Blood samples were collected from all patients and the control group for the detection of anti-*H pylori* IgG.

Detection of anti-*H pylori* IgG In order to eliminate possibly disrupted other proteins, each sample was diluted at 1:100 and detected in duplicate according to the manufacturer's instructions. A series of standard samples with concentrations of 0, 5, 10, 20, 35 and 70 units/ml were added in corresponding reactive wells. When the reaction was stopped, the optical density (OD) values were tested within 10 min at light wavelength 450nm. To measure the concentrations of anti-*H pylori*-IgG in the serum samples, a standardized curve of each board was mapped with the concentrations of standard samples as the abscissa, and OD values of the two correspondent parallel wells as the ordinate. The average OD value of each sample in the two parallel wells above 12 units/ml was regarded to be positive, otherwise to be negative.

Statistical analysis Data analysis was conducted with χ^2 test.

RESULTS

Detection of serum anti-H pylori IgG in patients with different digestive cancers

The positive rates of anti-*H pylori* IgG in patients with digestive cancers and healthy subjects were 61.50 % (230/374) and 46.77 % (145/310), respectively, which were significantly different ($P<0.01$). The positive rates were 52.38 % (33/63), 86.60 % (84/97), 83.14 % (84/101), 45.24 % (19/42), 51.13 % (18/35) and 44.44 % (16/36), respectively, in patients with esophageal carcinoma, gastric cancer, duodenal carcinoma,

rectal cancer, colon carcinoma and liver cancer. There was a significant difference (P<0.01). The detailed results are shown in Table 1.

Table 1 Positive rates of *H pylori*-IgG in patients with different peptic cancers

	n	Anti-*H pylori* IgG	
		-	+ (%)
Normal group	310	165	145 (46.77)[a]
Cancer group	374	144	230 (61.50)[a]
Esophagus carcinoma	63	30	33 (52.38)[b]
Gastric cancer	97	13	84 (86.60)[b]
duodenal Carcinoma	101	17	84 (83.17)[b]
Rectal cancer	42	23	19 (45.24)[b]
Carcinoma of colon	35	17	18 (51.43)[b]
Liver cancer	36	20	16 (44.44)[b]

[a]P<0.01, χ^2=14.8; [b]P<0.01, χ^2=58.69.

Serum anti-H pylori IgG in patients with gastric cancer

The positive rates of anti-*H pylori* IgG in intestinal and diffuse type gastric cancer were 93.75 % (60/64) and 72.73 % (24/33), respectively (P<0.05).In addition, the positive rates in gastric antrum and cardia were 96.43% (54/56) and 73.17% (30/41) (P<0.05). The detailed results are shown in Table 2.

Table 2 Positive rates of anti-*H pylori* IgG in patients with gastric cancer of different types and at different locations

Group	*n*	*H pylori*-IgG	
		Positive	Positive rates (%)
Gastric cancer	97	84	86.60
Intestinal type	64	60	93.75[c]
Diffuse type	33	24	72.73[c]
Gastric antrum	56	54	96.43[d]
Gastric cardiac	41	30	73.17[d]

[c]P<0.05, χ^2=8.29; [d]P<0.05, χ^2=11.03.

Serum anti-H pylori IgG in patients with duodenal carcinoma

The positive rates of anti-*H pylori* IgG in patients with ulcerous and proliferous type duodenal carcinoma were 91.04 % (61/67) and 52.27 % (23/44) (P<0.05). In addition, the positive rates of *H pylori*-IgG in the bulb and descending part of duodenum were 94.20 % (65/69) and 45.20 % (19/42), (P<0.05). The detailed results are shown in Table 3.

Table 3 Positive rates of *H pylori*-IgG in patients with duodenal carcinoma of different types and at different locations

Group	*n*	*H pylori*-IgG	
		Positive	Positive rates (%)
Duodenal carcinoma	101	84	83.17
Ulcerous type	67	61	91.04[e]
Proliferous type	44	23	52.27[e]
Bulb of duodenum	69	65	94.20[f]
Descending part	42	19	45.20[f]

[e]P<0.05, χ^2=19.74; [f]P<0.05, χ^2=28.97.

DISCUSSION

H pylori is one of the common bacteria causing chronic infection, infects more than 50 % of the human population, causes chronic gastritis and plays an important role in the pathogenesis of gastroduodenal ulceration. *H pylori* has also been suggested to be involved in the genesis of adenocarcinoma and MALT lymphoma of the stomach[11-13]. It is believed that *H pylori* infection might result in the release of various bacterial and host dependent cytotoxic substances including ammonia, platelet activating factor, cytotoxins and lipopolysaccharide as well as cytokines such as interleukins (IL)-1-12, tumor necrosis factor alpha (TNF-alpha) and reactive oxygen species[14-20], tissue damage and gastro-duodenal disease[21-25]. In 1994, the World Health Organization and International Agency for Research on Cancer (IARC) classified it as a class I carcinogen[26-28]. In this study sera from 374 patients with digestive cancers and 310 healthy controls were tested for *H pylori* using a specific IgG ELISA. The results showed that the positive rate of anti-*H pylori* IgG was 61.50 % in the patients, which was significantly greater than that (46.77 %) in the control group (P<0.01). This finding indicated that patients with digestive cancers were more susceptible to infection by *H pylori* than healthy subjects, which might be related to a lower immunity in these patients. Furthermore, there was a significant difference in the positive rate among patients with cancers of esophageal, stomach, duodenum, rectum, colon and liver. This observation indicated that the prevalence of *H pylori* infection in patients with different digestive cancers was different, which was significantly higher in gastric and duodenal carcinomas than in other digestive cancers. All of these results were concordant with those previously reported[29-34].

In this study, the infection rate was 86.60 % (84/97) in patients with gastric cancer, with a rate of 96.43 % in antral cancer and 93.75 % in intestinal type cancer. We postulate that *H pylori* infection plays an important role in carcinomatous changes in gastric antrum, and is an important pathogenic factor causing intestinal type gastric cancer. This notion is consistent with previous literatures[35-39]. The histological process of intestinal type gastric cancer has been described as normal gastric mucosa→superficial chronic gastritis→atrophic gastritis→intestinal metaplasia→atypical hyperplasia→gastric cancer[40]. After long-term infection of *H pylori* in gastric mucosa, secretion of gastric acid could be reduced, flora in intestinal tract might survive and breed in stomach, and some bacteria recovering nitrate salts might form N-nitroso compounds that are important carcinogens. Moreover, *H pylori* leads to a decrease of vitamin C, which is a strong antioxidant and protective factor in gastric juice, preventing against the occurrence of gastric cancer. As a result, the levels of reactive oxygen and free radicals would increase, and direct DNA damage would incur. Thus, the chances of gene mutation would increase, and further accelerate the development of gastric cancer[41,42].

At present, the definite etiological factors of duodenal carcinoma are not clear, although many studies have suggested that some cholic acids like deoxycholic acid and its degradation products be related to the occurrence and development of duodenal carcinoma. Additionally, ulcerous and genetic factors have been considered to be associated with duodenal carcinoma. Stromberg *et al* found that the levels of several cytokines, such as interleukin-8 (IL-8), transforming growth factor beta (TGF-beta) and gamma interferon (IFN-gamma), were significantly lower in duodenal ulcer (DU) patients than in asymptomatic carriers (AS) and uninfected individuals. Then it was suggested that a number of cytokines might be important for the mucosal host defense against *H pylori* and a down-regulated immune response would play a role in the development of duodenal ulcers[43]. Colonizing in gastric antrum, *H pylori* can destroy the inhibitory feedback adjustment of gastrin release, which results in increased acid load in

duodenum, raises the risk of impairment of duodenal mucous membrane and thus convering of duodenal mucosa to gastric metaplasia. The metaplastic epithelium could provide a site where *H pylori* colonize, and cause duodenitis that was pre-ulcer status of DU and formed ulcer in the end[44]. In addition, some studies have suggested that the development of DU is related to *H pylori* density in patients. There was a tendency of higher *H pylori* density when the degree of deformity of the duodenal bulb increased[45]. The results of our study showed that 83.17 % of the patients with duodenal carcinoma were infected by *H pylori*, with the rate being 91.04 % in ulcerous type and 94.20 % in the bulb carcinoma. Therefore, we conclude that *H pylori* infection is associated with the development of duodenal carcinoma, especially with ulcerous type and in duodenal bulb.

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