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

Ke-Xia Wang, Xue-Feng Wang, Jiang-Long Peng, Yu-Bao Cui, Jian Wang, Chao-Pin Li

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 % (175/374), 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.

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 \(\chi^2\) 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 % (175/374), 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**

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

\[ \chi^2 < 0.01, \chi^2 = 14.8; \chi^2 < 0.05, \chi^2 = 58.69. \]

**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 |
| Diffuse type               | 33  | 24       | 72.73 |
| Gastric antrum             | 56  | 54       | 96.43 |
| Gastric cardiac            | 41  | 30       | 73.17 |

\[ \chi^2 < 0.05, \chi^2 = 8.29; \chi^2 < 0.05, \chi^2 = 11.03. \]

**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 |
| Proliferous type           | 44  | 23       | 52.27 |
| Bulb of duodenum           | 69  | 65       | 94.20 |
| Descending part            | 42  | 19       | 45.20 |

\[ \chi^2 < 0.05, \chi^2 = 19.74; \chi^2 < 0.05, \chi^2 = 28.97. \]

**DISCUSSION**

\(H\) pylori is one of the common bacteria causing chronic gastritis, 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. 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, cytokotnics and lipopolysaccharide as well as cytokines such as interleukins (IL)-1-12, tumor necrosis factor alpha (TNF-alpha) and reactive oxygen species, tissue damage and gastro-duodenal disease. In 1994, the World Health Organization and International Agency for Research on Cancer (IARC) classified it as a class I carcinogen. 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.

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. 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. 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.

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. 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 mucosal membrane and thus convering of duodenal mucosa to gastric metaplasia. The metaplastic epithelium could provide a site where Helicobacter pylori colonize, and cause duodenitis that was pre-ulcer status of DU and formed ulcer in the end[41]. In addition, some studies have suggested that the development of DU is related to Helicobacter pylori density in patients. There was a tendency of higher Helicobacter pylori density when the degree of deformity of the duodenal bulb increased[42]. The results of our study showed that 83.17% of the patients with duodenal carcinoma were infected by Helicobacter pylori, with the rate being 91.04% in ulcer type and 94.20% in the bulb carcinoma. Therefore, we conclude that Helicobacter pylori infection is associated with the development of duodenal carcinoma, especially with ulcerous type and in duodenal bulb.

ACKNOWLEDGEMENTS

We thank Department of Pathology, Benbu Medical College, Department of Oncology, Huainan First Miner’s Hospital and Department of Oncology, Huainan Second Miner’s Hospital, as well as Department of Oncology, Huainan Third Miner’s Hospital for sample collection.

REFERENCES

1 Oyejide KS, Smith SI, Arigbabu AO, Coker AO, Nububa DA, Agbakwuru EA, Atoyebi OA. Use of direct Gram stain of stomach biopsy as a rapid screening method for detection of Helicobacter pylori from peptic ulcer and gastritis patients. J Basic Microbiol 2002; 42: 121-125
2 Nguen TM, Barkun AN, Fallone CA. Host determinants of Helicobacter pylori infection and its clinical outcome. Helicobacter 1999; 4: 185-197
3 Brigi E, Hodzic L, Zldicz M. Helicobacter pylori and gastroduodenal disease in our patients: 2-year experience. Med Arh 2000; 54: 313-316
4 Zawilaak A, Zakrzewska-Czerwinska J. Organization of the Helicobacter pylori genome. Postepy Hig Med Dosw 2001; 55: 357-361
5 Vandeplas Y. Helicobacter pylori infection. World J Gastroenterol. 2000; 6: 20-31
6 McNamara D, O’ Morain C. Helicobacter pylori and gastric cancer. Ital J Gastroenterol Hepatol 1998; 30: 294-298
7 Pineros DM, Riveros SC, Marin JD, Ricardo O, Diaz OO. Helicobacter pylori in gastric cancer and peptic ulcer disease in a Colombian population. Strain heterogeneity and antibody profiles. Helicobacter 2001; 6: 199-206
8 Tanida N, Sakagami T, Nakamura Y, Kawaura A, Hikasa Y, Shimoyama T. Helicobacter pylori and gastric cancer. Nippon Geka Gakkai Zasshi 1996; 97: 257-262
9 Hira M, Azuma T, Ito S, Kato T, Kohi Y, Fujiki Y. High prevalence of neutralizing activity to Helicobacter pylori cytotoxin in serum of gastric-carcinoma patients. Int J Cancer 1994; 56: 56-60
10 Queiroz DM, Mendes EN, Rocha GA, Oliveira AM, Oliveira CA, Cabral MM, Nogueira AM, Souza FA. Serological and direct diagnosis of Helicobacter pylori in gastric carcinoma: a case-control study. J Med Microbiol 1999; 48: 503-506
11 Nakajima M, Kuwayama H, Ito Y, Iwasaki A, Arakawa Y. Helicobacter pylori, neutrophils, interleukins, and gastric epithelial proliferation. J Clin Gastroenterol 1997; 25: 198-202
12 Sato K, Sugano K. Causal relationship between Helicobacter pylori infection and upper gastroduodenal diseases. Nippon Rinsho 2001; 59: 239-245
13 Muller S, Sefert E, Stolte M. Simultaneous MALT-type lymphoma and early adenocarcinoma of the stomach associated with Helicobacter pylori gastritis. Z Gastroenterol 1999; 37: 153-157
14 Ramirez Ramos AA, Helicobacter pylori. Rev Gastroenterol Peru 2001; 21: 99-101
15 Yoshimura N, Suzuki Y, Saito Y. Suppression of Helicobacter pylori-induced interleukin-8 production in gastric cancer cell lines by an anti-ulcer drug, geranylgeranylacetone. J Gastroenterol Hepatol 2001; 17: 1153-1160
16 Stass G, Arena A, Speranza A, Iannello D, Mastroeni P. Different modulation by live or killed Helicobacter pylori on cytokine production from peripheral blood mononuclear cells. New Microbiol 2002; 25: 247-252
17 Ji KY, Hu PL. Progress on Helicobacter pylori and cytokine. Shijie Xieyi Xiabua Zahi 2002; 10: 503-506
18 Walkier MM. Cytochrome-oxygenase-2 expression in early gastric cancer, intestinal metaplasia and Helicobacter pylori infection. Eur J Gastroenterol Hepatol 2002; 14: 347-349
19 Konturek PC, Konturek SJ, Bielsanski W, Karczewska E, Pierzchalski P, Duda A, Starzynska T, Marlicz K, Popiela T, Hartwich A, Hahm E. Role of gastrin in gastric cancerogenesis in Helicobacter pylori infected humans. J Physiol Pharmacol 1999; 50: 857-873
20 Han FC, Yan XJ, Su C. Expression of the CagA gene of Helicobacter pylori and application of its product. World J Gastroenterol 2000; 6: 122-124
21 Recaravene Ascencios R, Recaravene Arse S. Chronic atrophic gastritis: pathogenic mechanisms due to cellular hypersensitivity. Rev Gastroenterol Peru 2002; 22: 199-205
22 Al-Muhtaseb MH, Abu-Khalaf AM, Augheenaa AA. Ultrastructural study of the gastric mucosa and Helicobacter pylori in duodenal ulcer patients. Saudi Med J 2001; 20: 569-573
23 Zhang ZW, Farning MJ. Molecular mechanisms of Helicobacter pylori associated gastric carcinogenesis. World J Gastroenterol 1999; 5: 369-374
24 Naito Y, Yoshikawa T. Molecular and cellular mechanisms involved in Helicobacter pylori-induced inflammation and oxidative stress (1,2). Free Radic Biol Med 2002; 33: 323-326
25 Allen LA. Intracellular niches for extracellular bacteria: lessons from Helicobacter pylori. J Leukoc Biol 1999; 66: 753-756
26 Xue FB, Xu YY, Wang Y, Pan BR, Ren J, Fan DM. Association of Helicobacter pylori infection with gastric carcinoma: a meta analysis. World J Gastroenterol 2001; 7: 801-804
27 Palatka K, Altorjy S, Szakall S, Gyorffy A, Udvardy M. Detection of Helicobacter pylori in tissue samples of stomach cancer. Orv Hetil 1999; 140: 1985-1986
28 Mihelieks S, Kirsch C, Dragosics B, Gschwantler M, Oberhuber G, Antos D, Dite P, Lauter J, Leodolter A, Malfertheiner P, Neubauer A, Ehninger G, Stolte M, Bayerdorffer E. Helicobacter pylori and gastric cancer: current status of the Austrain Czech German gastric cancer prevention trial (PRISMA Study). World J Gastroenterol 2001; 7: 243-247
29 Lan J, Jiang YY, Lin YX, Wang BC, Gong LL, Xu HS, Guo GS. Helicobacter pylori infection generated gastric cancer through p53-Rb tumor-suppressor system mutation and telomere reactivation. World J Gastroenterol 2003; 9: 54-58
30 Wu AH, Crabtree JE, Bernstein L, Hawthip M, Tsang CC, Forman D. Role of Helicobacter pylori CagA+ strains and risk of adenocarcinoma of the stomach and esophagus. Int J Cancer 2007; 121: 815-821
31 Kuniyasu H, Yasui W, Yokozaki H, Tahara E. Helicobacter pylori infection and carcinogenesis of the stomach. Langenbecks Arch Surg 2000; 385: 69-74
32 Meining A, Bayerdorffer E, Muller P, Mihelieks S, Lehn N, Holzel D, Hatzk R, Stolte M. Gastric carcinoma risk index in patients infected with Helicobacter pylori. Virchows Arch 1998; 432: 311-314
33 Kuijpers EJ, Gracia-Casanova M, Pena AS, Pals G, Van Kamp G, Kok A, Kurz-pohlmann E, Pels NF, Meuwissen SG. Helicobacter pylori serology in patients with gastric carcinoma. Scand J Gastroenterol 1998; 32: 433-437
34 Rudelli A, Viallette G, Brazier F, Seraulut PC, Capron D, Dupas L. Helicobacter pylori and gastroduodenal disorders in 547 symptomat young adults. Gastroenterol Clin Biol 1996; 20: 367-373
35 Farinati F, Valante F, Germana B, Della Libera G, Baffa R, Rugge M, Piebani M, Vianello F, Di Mario F, Naccarato R. Prevalence of Helicobacter pylori infection in patients with precancerous changes of gastric cancer. Eur J Cancer Prev 1993; 2: 321-326
36 Meining AG, Bayerdorffer E, Stolte M. Helicobacter pylori gastritis of the gastric cancer phenotype in relative of gastric carcinoma patients. Eur J Gastroenterol Hepatol 1999; 11: 712-720
37 Zilmaity HM, Ota H, Graham DY, Akamatsu T, Katsuyama T.
Patterns of gastric atrophy in intestinal type gastric carcinoma.
Cancer 2002; 94: 1428-1436

38 Vasilenko IV, Surgai NN, Sidorova ID. Modifications of gastric mucosa in diffuse and intestinal cancer. Arkh Patol 2001; 63: 26-30

39 Xia HH, Kalantar JS, Talley NJ, Wyatt JM, Adams S, Chueng K, Mitchell HM. Antral-type mucosa in the gastric incisura, body, and fundus (antralization): a link between Helicobacter pylori infection and intestinal metaplasia. Am J Gastroenterol 2000; 95: 114-121

40 Meining A, Morgner A, Miehlke S, Bayerdorffer E, Stolte M. Atrophy-metaplasia-dysplasia-carcinoma sequence in the stomach: a reality or merely an hypothesis? Best Pract Res Clin Gastroenterol 2001; 15: 983-998

41 Xia HH, Talley NJ. Apoptosis in gastric epithelium induced by Helicobacter pylori infection: implications in gastric carcinogenesis. Am J Gastroenterol 2001; 96: 16-26

42 Pignatelli B, Bancel B, Esteve J, Malavelle C, Carmels S, Correa P, Patricot LM, Laval M, Lyandrat N, Ohshima H. Inducible nitric oxide synthase, anti-oxidant enzymes and Helicobacter pylori infection in gastritis and gastric precancerous lesion in humans. Eur J Cancer Prev 1998; 7: 439-447

43 Kuipers EJ, Thijs JC, Feston HP. The prevalence of Helicobacter pylori infection in peptic ulcer disease. Aliment Pharmacol Ther 1995; 9: 59-69

44 Olbe L, Hamlet A, Dalenback J, Fandriks L. A mechanism by which Helicobacter pylori infection of the antrum contributes to the development of duodenal ulcer. Gastroenterology 1996; 110: 1386-1394

45 Lai YC, Wang TH, Liao CC, Huang SH, Wu CH, Yang SS, Lee CL, Chen TK. Correlation between the degree of duodenal bulb deformity and the density of Helicobacter pylori infection in patients with active duodenal ulcers. J Formos Med Assoc 2002; 101: 263-267

Edited by Xia HHX and Wang XL