Rapid communication

Evaluation of the role of *H pylori* infection in pathogenesis of gastric cancer by immunoblot assay

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Abstract

AIM: To elucidate the different serological reactions to *H pylori* using the immunoblotting technique for further understanding of its pathogenic role in gastric cancer.

METHODS: A total of 54 patients were divided into two groups after upper gastrointestinal endoscopy: normal control group (25 patients) and gastric cancer group (29 patients). Both groups were further divided into *H pylori* (+) and *H pylori* (-) subgroups based on the results of CLO test, Giemsa staining and culture. Sera were further analyzed with the immunoblotting technique (HelicoBlot 2.0, Genelabs Diagnostics, Singapore).

RESULTS: The positive rate of the immunoblotting test was as high as 88.9% in the *H pylori* (-) gastric cancer group and only 14.3% in the *H pylori* (-) normal control group with a statistically significant difference.

CONCLUSION: The prevalence of *H pylori* infection is higher in gastric cancer patients than in the normal controls, suggesting that *H pylori* may play a role in the pathogenesis of gastric cancer.

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Key words: Western blot; Immunoblotting; Gastric cancer; *H pylori*; Enzyme-linked immunosorbent assay

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INTRODUCTION

*H pylori*, a Gram-negative bacterium, is now widely considered as one of the major etiologic factors in the pathogenesis of a great variety of gastrointestinal diseases such as gastritis, peptic ulcers and mucosa-associated lymphoid tissue lymphomas (MALTomas)[1]. There is increasing evidence that cancer of the stomach is also strongly associated with *H pylori* colonization[2-6]. Numerous antibodies against antigens of *H pylori* can be detected by serological analysis using the Western immunoblot technique[7-10]. Among these antibodies to *H pylori*, polypeptides with molecular masses of 116 kDa (against the cytotoxin-associated antigen, CagA), 89 kDa (against the vacuolating toxin antigen, VacA), 35 kDa, 30 kDa, 26.5 kDa and 19.5 kDa are considered as the most specific antibodies used in the diagnosis of *H pylori* infection and their corresponding antigens probably play a pathogenic role in the distinct gastrointestinal diseases. Particularly the antigens CagA and VacA not only seem to have a significant association with peptic ulcer disease but also increase the risk of developing gastric cancer[11-16]. The aim of this study was to elucidate the probable pathogenic role of *H pylori* in gastric cancer and serological stigmata of its remote infection as detected by the immunoblotting technique.

MATERIALS AND METHODS

Patients

Between March 1998 and May 2000, 54 consecutive patients (34 women, 20 men; age range: 20-70 years) who had epigastralgia and vague abdominal complaints but no remarkable past medical history of systemic diseases (such as generalized sepsis, uremia or hematologic malignancies) were recruited prospectively in this study. These patients visited the Outpatient Clinic or the Health Management Center of Shin Kong Wu Ho-Su Memorial Hospital for a routine health check-up. During upper GI endoscopy, specimens were taken from the antrum for rapid urease test, Giemsa stain and culture to elucidate the patient’s *H pylori* status. When gastric malignancy was suspected, more specimens were taken from the lesion for histological examination. The patients were then divided into a normal control group (*n* = 25) and a gastric cancer group (*n* = 29) (Table 1). The normal control group and gastric cancer group were further divided into *H pylori* (+) and *H pylori* (-) subgroups. The *H pylori* (+) subgroup had positive results in at least two of the three tests, while the three tests were
Table 1 Positive rate (%) of different reaction bands in the two groups of patients

|        | Normal | CA          |
|--------|--------|-------------|
|        | H pylori (+) | H pylori (-) | H pylori (+) | H pylori (-) | P          |
| n      | (11%) | (14%)       | (11%) | (18%)       |        |
| Overall| 100.0 | 14.3        | 100.0 | 88.9        | < 0.0001 |
| 116 kDa| 100.0 | 14.3        | 90.9  | 72.2        | 0.002    |
| 89 kDa | 44.4  | 0.0         | 60.0  | 27.8        | 0.052    |
| 35 kDa | 100.0 | 0.0         | 100.0 | 61.1        | 0.0003   |
| 30 kDa | 100.0 | 7.14        | 81.8  | 58.80       | 0.003    |
| 26.5 kDa| 90.9 | 14.3        | 72.7  | 66.7        | 0.005    |
| 19.5 kDa| 72.7 | 0.0         | 40.0  | 16.6        | 0.238    |

Fisher’s exact test was used to test for the different positive rate between CA-H pylori (+) and Normal-H pylori (-) group.

RESULTS

The seroprevalence of antibodies to 116 kDa (Cag-A), 89 kDa (VacA), 35 kDa, 30 kDa, 26.5 kDa and 19.5 kDa was quite similar finding was observed with the 35 kDa antigen. A

all negative in the H pylori (-) subgroup. In the H pylori (+) normal subgroup there were 12 female and 2 male patients with a mean age of 34.6 years. In the H pylori (+) normal subgroup there were 7 female and 4 male patients with a mean age of 37.2 years. In the H pylori (+) cancer subgroup there were 9 female and 9 male patients with a mean age of 58.8 years. In the H pylori (+) cancer subgroup there were 6 female and 5 male patients with a mean age of 59 years. In the H pylori (+) cancer subgroup, tumors were found in the gastric antrum, angle, corpus and cardia of 6, 3, 5 and 4 patients, respectively. Meanwhile, in the H pylori (+) cancer subgroup, tumors were found in the antrum of 5 patients (2 of them had tumor involving antrum and angle), in the antrum and lower corpus of 2 patients, in the angle of one patient, in the corpus of 3 patients, and in the corpus as well asfundus and cardia of one patient. Histopathological studies demonstrated that all the suspicious malignant lesions were adenocarcinoma. In order to analyze the possible link between gastric cancer and remote H pylori infection, the sera from patients were analyzed with the immunoblotting technique (HelicoBlot 2.0, Genelabs Diagnostics, Singapore) (Figure 1). Five reaction bands could be recognized with the immunoblot technique: 116 kDa (CagA), 89 kDa (VacA), 35 kDa, 30 kDa, 26.5 kDa and 19.5 kDa. The immunoblotting was considered as positive with the detection of one reaction band of 116 kDa (CagA) and/or 89 kDa (VacA) and/or 35 kDa (major antigens), and/or two other reaction bands (minor antigens, 30 kDa, 26.5 kDa, 19.5 kDa), as recommended by the manufacturer. In addition, sera from the H pylori (-) cancer group of patients were further analyzed by enzyme-linked immunosorbent assay (ELISA, Immulite H pylori IgG, Diagnostic Products Corporation, Los Angeles, USA), and the two serological methods were compared. The collected data were finally analyzed with the Fisher’s exact test.

The seroprevalence of antibodies to 116 kDa (Cag-A) positive H pylori strain was high among the patients enrolled in this study: 100% in the normal H pylori (+) control group, 90.9% in the CA-H pylori (+) group, and also strikingly high in the CA-H pylori (-) group (72.2%). A
cancer patients with their tumor localized in the cardia were excluded from statistical analysis, the overall result was identically significant (Table 2).

**DISCUSSION**

The role of different *H pylori* antigens in gastrointestinal diseases still remains controversial. In contrast to Western developed countries, different reaction bands in immunoblot assay fail to predict a particular disease in Taiwanese patients [17-21]. Two *H pylori* proteins, VacA and CagA, are virulence factors which may enhance gastric mucosal damage and promote the development of peptic ulcers and gastric mucosa atrophy. By identifying different *H pylori* proteins, immunoblot assay can screen patients at high risk of developing gastrointestinal diseases, such as peptic ulcer and gastric cancer. However, the high seroprevalence of antibodies to CagA-positive *H pylori* strains in Taiwanese patients with various gastrointestinal diseases has rendered the CagA-positive phenotype, an unusable marker for screening patients with a determined disease and immunoblot assay has no predictive and diagnostic value in Taiwanese patients. ELISA may reveal a significant decrease in IgG antibody titers approximately two months after treatment with antimicrobials. In contrast, immunoblot assay may detect IgG antibodies to specific antigens such as CagA and VacA several years after treatment [22-24]. These findings suggest that ELISA is a useful quantitative tool for monitoring eradication of *H pylori* while immunoblot assay is a qualitative method able to demonstrate remote *H pylori* infections which are not detectable by ELISA. The sensitivity and specificity of ELISA may decrease with the decrease in IgG titers. The immunoblotting technique might be recommended as a confirmative test for antibodies detected by ELISA [25,26]. Furthermore, although a high accuracy has been reported in Western countries, commercial ELISA might be unsatisfactory in Asians [27]. Therefore, immunoblot assay may be regarded as a sensitive, non-invasive means for the diagnosis of *H pylori* infection. However, major serological cross-reactions with *Campylobacter jejuni* and bacterial lipopolysaccharide have been found, which might explain the false positive results, while decrease in concentration of antibodies might yield equivocal reaction bands. It is known that *H pylori* colonization causes chronic active inflammation of gastric mucosa which eventually leads to the development of atrophic gastritis, intestinal metaplasia and dysplasia. Eighty-nine percent of *H pylori* (-) patients with gastric adenocarcinoma were proven to have a positive immunoblot assay in this study, indicating that these patients might have been infected with *H pylori* in a certain past period of their lifetime. This interesting finding suggests that *H pylori* can be detected in hostile gastric environments such as mucosa atrophy, but its hidden remote infection is still demonstrated in serum by immunoblot assay [28]. Therefore, the role of *H pylori* in the pathogenesis of gastric cancer should be stressed. Further studies are necessary to elucidate the possible link between *H pylori* infection and mechanisms of carcinogenesis.

In conclusion, 88.9% of patients with gastric cancer in *H pylori* (-) subgroup have a positive immunoblot assay for

| Table 2 Positive rate (%) of the different reaction bands in the two groups of patients |
|-----------------------------------------------|--------------------|-----------------|-----------------|------------------|
| n                                      | Overall (%) | 116 kDa (%) | 89 kDa (%) | 35 kDa (%) | 30 kDa (%) | 26.5 kDa (%) | 19.5 kDa (%) |
| CA (+) H pylori | 100.0 | 14.3 | 100.0 | 90.9 | 71.4 | 92.9 | <0.0001 |
| CA (-) H pylori | 100.0 | 14.3 | 100.0 | 90.9 | 71.4 | 92.9 | <0.0001 |

Four *H pylori* (+) patients with their tumors localized in the cardia were excluded from the analysis. Fisher’s exact test was used to test for the different positive rate between CA-*H pylori* (+) and Normal-*H pylori* (-) groups.

**REFERENCES**

1. Chang CS, Chen LT, Yang JC, Lin JT, Chang KC, Wang JT. Isolation of a Helicobacter pylori protein, FldA, associated with mucosa-associated lymphoid tissue lymphoma of the stomach. *Gastroenterology* 1999; 117: 82-88
2. Valle J, Gisbert JP. Helicobacter pylori infection and precancerous lesions of the stomach. *Hepatogastroenterology* 2001; 48: 1548-1551
3. Huang JQ, Sridhar S, Chen Y, Hunt RH. Meta-analysis of the relationship between Helicobacter pylori seropositivity and gastric cancer. *Gastroenterology* 1998; 114: 1169-1179
4. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med* 1991; 325: 1127-1131
5. Metz DC. Helicobacter pylori and gastric cancer: proving the link. *Am J Gastroenterol* 1999; 94: 852-853
6. Scheiman JM, Cutler AF. Helicobacter pylori and gastric cancer. *Am J Med* 1999; 106: 222-226
7. Holtmann G, Talley NJ, Mitchell H, Hassell S. Antibody response to specific *H. pylori* antigens in functional dyspepsia, duodenal ulcer disease, and health. *Am J Gastroenterol* 1998; 93: 1222-1227
8. Nilsson I, Ljungh A, Aleljung P, Wadström T. Immunoblot assay for serodiagnosis of Helicobacter pylori infections. *J Clin Microbiol* 1997; 35: 427-432
9. Aucker P, Petit ML, Mannant PR, Pezenne L, Babin P, Fauchere JL. Use of immunoblot assay to define serum antibody patterns associated with Helicobacter pylori infection and with *H pylori*-related ulcers. *J Clin Microbiol* 1998; 36: 931-936
10. Andersen LP, Killierick S, Pedersen G, Thoresen AC, Jergensen F, Rath J, Larsen NE, Barup O, Krogfelt K, Scheibel J, Rune S. An analysis of seven different methods to diagnose Helicobacter pylori infections. *Scand J Gastroenterol* 1998; 33: 24-30
11. Miehlke S, Go MF, Kim JG, Graham DY, Figura N. Serologic detection of Helicobacter pylori infection with cagA-positive strains in duodenal ulcer, gastric cancer, and asymptomatic gastritis. *J Gastroenterol* 1998; 33 Suppl 10: 18-21
12. Rudi J, Kolb C, Maiwald M, Zuna I, von Herbay A, Galle PR, Streumbled W. Serum antibodies against Helicobacter pylori proteins VacA and CagA are associated with increased risk for gastric adenocarcinoma. *Dig Dis Sci* 1997; 42: 1652-1659
13. Yamaoka Y, Kodama T, Graham DY, Kashima K. Search for putative virulence factors of Helicobacter pylori: the low-molec...

www.wig.net.com
ular-weight (33-35 K) antigen. *Dig Dis Sci* 1998; 43: 1482-1487

14. Vaucher C, Janvier B, Nousbaum JB, Grignon B, Pezennec L, Robaszkiewicz M, Gouerou H, Picard B, Fauchere JL. Antibody response of patients with *Helicobacter pylori*-related gastric adenocarcinoma: significance of anti-cagA antibodies. *Clin Diagn Lab Immunol* 2000; 7: 463-467

15. Beales IL, Crabtree JE, Scunes D, Covacci A, Calam J. Antibodies to CagA protein are associated with gastric atrophy in *Helicobacter pylori* infection. *Eur J Gastroenterol Hepatol* 1996; 8: 645-649

16. Yang H, Wu SV, Pichuantes S, Song M, Wang J, Zhou D, Xu Z, Quan S, Polito A, Walsh JH. High prevalence of cagA-positive strains in *Helicobacter pylori*-infected, healthy, young Chinese adults. *J Gastroenterol Hepatol* 1999; 14: 476-480

17. Yang JC, Wang TH, Wang HJ, Kuo CH, Wang JT, Wang WC. Genetic analysis of the cytotoxic-associated gene and the vacuolating toxin gene in *Helicobacter pylori* strains isolated from Taiwanese patients. *An J Gastroenterol* 1997; 92: 1316-1321

18. Shyu RY, Jiang SY, Lai CH, Hsu CT, Young TH, Yeh MY. High frequency of cytotoxic-associated gene A in Helicobacter pylori isolated from asymptomatic subjects and peptic ulcer patients in Taiwan. *J Clin Gastroenterol* 1998; 27: 54-59

19. Shiesh SC, Sheu BS, Yang HB, Tsao HJ, Lin XZ. Serologic response to lower-molecular-weight proteins of *H pylori* is related to clinical outcome of *H pylori* infection in Taiwan. *Dig Dis Sci* 2000; 45: 781-788

20. Wang JT, Sung CT, Lin JT, Wang TH. Helicobacter pylori in tumor tissues of patients with advanced gastric adenocarcinoma: high prevalence but failure to detect integration. *Zhonghua Bingdu Weisheng Wujimian YiXue Zazhi* 1996; 29: 134-142

21. Wang JT, Chang CS, Lee CZ, Yang JC, Lin JT, Wang TH. Antibody to a Helicobacter pylori species specific antigen in patients with adenocarcinoma of the stomach. *Biochem Biophys Res Commun* 1998; 244: 360-363

22. Sörberg M, Engstrand L, Ström M, Jönsson KA, Jörbeck H, Granström M. The diagnostic value of enzyme immunoassay and immunoblot in monitoring eradication of *Helicobacter pylori*. *Scand J Infect Dis* 1997; 29: 147-151

23. Kist M, Strobel S, Kirchner T, Dammann HG. Impact of ELISA and immunoblot as diagnostic tools one year after eradication of *Helicobacter pylori* in a multicentre treatment study. *FEMS Immunol Med Microbiol* 1999; 24: 239-242

24. Karvar S, Karch H, Frosch M, Burghardt W, Gross U. Use of serum-specific immunoglobulins A and G for detection of *Helicobacter pylori* infection in patients with chronic gastritis by immunoblot analysis. *J Clin Microbiol* 1997; 35: 3058-3061

25. Andersen LP, Espersen F. Immunoglobulin G antibodies to *Helicobacter pylori* in patients with dyspeptic symptoms investigated by the western immunoblot technique. *J Clin Microbiol* 1992; 30: 1745-1751

26. Klaamas K, Held M, Wadström T, Lipping A, Kurtenkov O. IgG immune response to *Helicobacter pylori* antigens in patients with gastric cancer as defined by ELISA and immunoblotting. *Int J Cancer* 1996; 67: 1-5

27. Leung WK, Ng EK, Chan FK, Chung SC, Sung JJ. Evaluation of three commercial enzyme-linked immunosorbent assay kits for diagnosis of Helicobacter pylori in Chinese patients. *Diagn Microbiol Infect Dis* 1999; 34: 13-17

28. Kerns WE Jr, Samloff IM, Siurala M, Kekki M, Sipponen P, Kim SW, Walsh JH. Positive serum antibody and negative tissue staining for Helicobacter pylori in subjects with atrophic body gastritis. *Gastroenterology* 1991; 101: 167-174