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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.

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* (-)	
n	11 (%)	14 (%)	11 (%)	18 (%)	*P*
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.

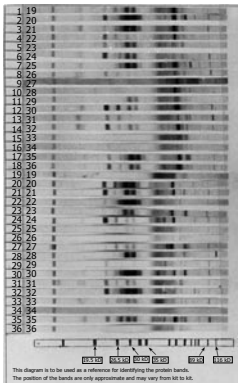

Figure 1 Example of the immunoblotting reaction bands. A reaction sheet from a group of patients enrolled in the study.

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 as fundus 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.

RESULTS

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 quite similar finding was observed with the 35 kDa antigen (Table 1). The seroprevalence of antibodies to the third major antigen 89 kDa (VacA) was 44.4% in the normal *H pylori* (+) group, 60% in the CA-*H pylori* (+) group and 35.7% in the CA-*H pylori* (-) group. In the case of minor antigens, the seroprevalence of antibodies to the 30 kDa antigen was 100% in the normal *H pylori* (+) group, 81.8% in the CA-*H pylori* (+) group, and also remarkably high (58.8%) in the CA-*H pylori* (-) group. For the 26.5 kDa antigen, the seroprevalence of antibodies was 90.9% in the normal *H pylori* (+) group, 72.7% in the gastric cancer-*H pylori* (+) group, and 66.7% in the CA-*H pylori* (-) group. For the 19.5 kDa antigen, the seroprevalence of antibodies was 72.7% in the normal *H pylori* (+) group, and 40% in the gastric cancer-*H pylori* (+) group. When the reaction bands were equivocal (neither positive nor negative), they were considered undetermined with a prevalence of 2.2% in the 116 kDa antigen, 10.9% in the 89 kDa antigen, 2.2% in the 35 kDa antigen, 8.7% in the 30 kDa antigen, 3.6% in the 26.5 kDa antigen, and 5.8% in the 19.5 kDa antigen. These equivocal reaction bands might indicate that a low serum concentration of the corresponding antibodies was insufficient to yield a clear-cut reaction with their respective antigens. Analysis of the seroprevalence of antibodies to different antigens yielded the following overall positive rates for immunoblotting test: 100% in the normal *H pylori* (+) subgroup, 14.3% in the normal *H pylori* (-) subgroup, 100% in the gastric cancer-*H pylori* (+) subgroup and 88.9% in the gastric cancer-*H pylori* (-) subgroup, respectively. It should be pointed out that the positive rate for the immunoblotting technique was strikingly higher in the gastric cancer-*H pylori* (-) subgroup than in the normal *H pylori* (-) subgroup and there was a statistically significant difference achieved by Fisher's exact test ($P < 0.05$). This interesting finding denoted that the presence of *H pylori* as a remote infection in both *H pylori* (-) subgroups detected by immunoblotting assay was much more significant in the gastric cancer-*H pylori* (-) subgroup than in the normal *H pylori* (-) subgroup. This important issue might be overlooked if only rapid urease test, Giemsa staining and culture were performed. However, when ELISA was carried out to detect IgG to *H pylori* antigens using sera from these 18 gastric cancer-*H pylori* (-) patients, only 9 of them were positive (50% *vs* 88.9%). Since *H pylori* might not be closely implicated in the development of tumors in the cardiac region, if the four *H pylori* (-)

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 *H pylori* infection. Immunoblot assay can disclose remote *H pylori* infection which might be overlooked if only rapid urease test, Giemsa staining and culture, or ELISA for IgG antibody, is performed.

Table 2 Positive rate (%) of the different reaction bands in the two groups of patients

	Normal		CA		
	H pylori (+)	*H pylori* (-)	*H pylori* (+)	*H pylori* (-)	
n	11 (%)	14 (%)	11 (%)	14 (%)	*P*
Overall	100.0	14.3	100.0	92.9	< 0.0001
116 kDa	100.0	14.3	90.9	71.4	0.006
89 kDa	44.4	0.0	60.0	35.7	0.04
35 kDa	100.0	0.0	100.0	57.1	0.002
30 kDa	100.0	7.14	81.8	75.1	0.01
26.5 kDa	90.9	14.3	72.7	71.4	0.006
19.5 kDa	72.7	0.0	40.0	14.3	0.482

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.

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