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Anti-*Helicobacter pylori* immunoglobulin G (IgG) and IgA antibody responses and the value of clinical presentations in diagnosis of *H. pylori* infection in patients with precancerous lesions

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Abstract

AIM: To determine the prevalence of *Helicobacter pylori* (*H. pylori*) infection, the serum anti-*H. pylori* immunoglobulin G (IgG) and IgA antibody responses, and the value of clinical presentations in diagnosis of *H. pylori* infection in patients with gastric atrophy, intestinal metaplasia and dysplasia.

METHODS: *H. pylori* infection was detected by histology in 209 patients with mild chronic atrophic gastritis (CAG, n=76), severe CAG (n=22), mild intestinal metaplasia (IM, n=22), severe IM (n=58), or dysplasia (DYS, n=31). Serum anti-*H. pylori* IgG and IgA were double sampled and evaluated by enzyme-linked immunoadsordent assays. 35 clinical presentations were observed and their relationship with *H. pylori* infection was analyzed by the *k*-means cluster method.

RESULTS: Both IgG and IgA levels in *H. pylori* positive patients were significantly higher than those negative for *H. pylori* (P<0.001-0.01). The prevalence of *H. pylori* was highest in severe IM (84.5 %), and lowest in mild CAG (51.3 %) (P<0.01). They were similar in severe CAG (68.2 %), mild IM (72.7 %), and DYS (67.7 %). In *H. pylori* positive patients, the IgG levels in severe CAG were significantly higher than those in mild CAG (P<0.01). In *H. pylori* negative patients, both IgG and IgA levels increased remarkably in severe IM, compared to those in mild IM (P<0.01-0.05). *H. pylori* infection exhibited no association with patient's gender (62.1 % in males; 71.7 % in females) and age (r=0.0814, P=0.241). The diagnostic accuracy based on 35 clinical presentations was 65.7 %. It could be improved by 5.7 % when only the assemblage of digestive symptoms were engaged, or by 8.6 % when the pathogenic factors, general status and grossoscopy were combined. The diagnostic accuracy could be decreased when only the general symptoms were engaged, or when the pathogenic factors were accompanied with some common digestive symptoms.

CONCLUSION: *H. pylori* infection is a major risk factor for the process from atrophy, IM to DYS of gastric mucosa. Serum IgG and IgA are good indicators to evaluate this progress with a certain arrearage. Investigation on the effective assemblages of clinical presentations may provide a better understanding in the pathogenesis, diagnosis and treatment for *H. pylori* infection.

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INTRODUCTION

The persistence or repeated infection of pathogenic factors in the stomach may result in the chronic process of gastritis with glandular atrophy (AT), intestinal metaplasia (IM), dysplasia (DYS) and so on at different stages, which indicates diversiform prognosis. The roles of immune reactions in *Helicobacter pylori* (*H. pylori*) pathogenesis and chronic gastritis (CG) are research areas of rapid progress[1-4]. It is now recognized that the clinical detection of serum antibody is effective in monitoring the *H. pylori* infection[5,6]. However, *H. pylori* infection and the levels of serum anti-*H. pylori* immunoglobulin antibodies at different stages of CG are not fully investigated. Moreover, the complex clinical manifestations of *H. pylori* infection and associated CG leads to a diagnostic and therapeutic dilemma for CG[7].

To determine the prevalence of *H. pylori* infection, the serum anti-*H. pylori* immunoglobulin G (IgG) and IgA antibody responses, and the value of clinical presentations in diagnosis of *H. pylori* infection in patients with gastric atrophy, intestinal metaplasia and dysplasia a population-based investigation was designed and a novel analytic method was proposed in this work. The study also took a different perspective in assessing the association between *H. pylori* infection and clinical presentations.

MATERIALS AND METHODS

Patients

A total of two hundred and nine patients with chronic gastritis, who were diagnosed through gastroscopy and mucosal biopsy, were included in the present study. All patients, who resided in Shandong province, were investigated by the Institute of Basic Theory, Chinese Academy of Traditional Chinese Medicine from 1999 to 2001. Among them 103 were males and 106 were females, aged from 45 to 72 with a mean age of 55 years old. Gastric biopsies were histologically evaluated for activity and chronicity of gastritis, and the presence of AT and/or IM according to the criterion of the visual analogue scale in Sydney classification and grading of gastritis[8]. The patients consisted of 76 with mild chronic atrophic gastritis (CAG), 22 with severe (CAG), 22 with mild IM, 58 with severe IM and 31 with DYS accompanied with mild IM. All patients had not received any anti- *H. pylori* treatment.

Diagnosis of H. pylori infection

Two hundred and nine specimens of gastric mucosa were obtained from each patient via endoscopy. Gastric mucosa was sampled from the area of greater curvature at gastric antrum, and *H. pylori* was determined by pathological staining with hematoxylin and eosin (HE) followed by Giemsa staining. Under microscope, *Helicobacter-like organisms* can be identified as a typical curve like S or C. They look like a short bacilli or globular body with a slight curve.

Detection of anti-H. pylori IgG and IgA antibodies

Blood was sampled twice from patients. Enzyme-linked immunoadsorbent assays (ELISA) were used to detect the levels of serum anti-*H. pylori* IgG and IgA antibodies. The test kits for the detection of anti-*H. pylori* -IgG and anti-*H. pylori* -IgA were purchased from Bioseed Co., USA. The value of the optical density (OD) was read by a microtiter plate reader at 450 nm.

Clinical presentations observation

35 clinical presentations were observed as follows: (1) Symptoms of the digestive system including appetite, distending fullness in the stomach, stomachache, distending fullness in the abdomen, pain in the hypochondrium, pain in the abdomen, singultus, nausea, vomit, acid regurgitation and epigastric upset, and heartburn; (2) General status including ear, eye, physique, complexion, stool, urine, oropharynx, taste, swollen, head, limbs, chest, hand and foot; (3) Spirit and psychological status including spirit, sleep, and emotion; (4) Glossoscopy including quality of tongue, body of tongue, and fur of tongue and (5) Pathogenic factors including smoking, alcohol, dietary bias, and dietary regularity. Each investigated symptom consisted of two to four subordinate items.

Statistical analysis

A SPSS 10.0 statistical package program was used for data analysis. The variables were processed by chi-square test, student' s *t* test, ANVOA analysis, and bivariate correlate analysis, where appropriate.

k-means cluster analysis

The *k*-means cluster analysis method has been applied in many areas including data mining, statistics, biology, and machine learning, and so on[9]. In this study, the relationship between the *H. pylori* infection and the 35 clinical presentations of patients was analyzed by this method. The diagnostic accuracy was obtained by the *k*-means algorithms. The distance between clusters was further verified by an ANOVA test.

RESULTS

As shown in Table 1, the detected positive rates of *H. pylori* varied were 72.7 % (16/22) in mild IM, 84.5 % (49/58) in severe IM, 67.7 % (21/31) in DYS, 51.3 % (39/76) in mild CAG, and 68.2 % (15/22) in severe CAG, respectively. Analyses of chi-square test showed that the detected positive rates of *H. pylori* were similar in mild IM, mild CAG, DYS, and severe CAG groups. The *H. pylori* positive rate in severe IM group was statistically higher than those in other groups (all $P<0.01$).

Table 2 shows the detective positive rates of *H. pylori* for the CG patients in relation to gender and age. There was no significant difference between the male (62.1 %, 64/103) and the female patients (71.7 %, 76/106) ($P>0.05$). The *H. pylori* infection rate was highest in the ages from 42 to 49 (59/80, 73.8 %). However, the ANVOA analysis showed that there was no significant difference among four groups with different ages ($P>0.05$). Bivariate correlation analysis also indicated that the detected positive rate of *H. pylori* was not remarkably associated with the age of patients ($r=0.0814$, $P=0.241$).

Table 3 lists the levels of serum anti- *H. pylori* IgG and IgA at different statge of CG. Both serum anti- *H. pylori* IgG and IgA levels were remarkably higher in *H. pylori* positive patients than those negative for *H. pylori* ($P<0.001$-0.01). In *H. pylori* positive patients the IgG level was significantly greater in severe CAG than in mild CAG ($P<0.01$). In *H. pylori* negative patients both the serum IgG and IgA levels increased significantly in severe IM, compared to those in mild IM ($P<0.01$-0.05). There was no significant difference in IgG and IgA levels between mild and severe IM in the presence of *H. pylori* infection ($P>0.05$).

Table 1 The detected positive rates of *H. pylori* at different stages of CG

H. pylori	Mild CAG	Severe CAG	Mild IM	Severe IM	DYS	Total
Number	76	22	22	58	31	209
Positive %	39 (51.3%)	15 (68.2%) [a]	16 (72.7%)	49 (84.5%)[b]	21 (67.7%)	140
Negative %	37 (48.7%)	7 (31.8%)	6 (27.3%)	9 (15.5%)	10 (32.3%)	69

CAG, chronic atrophic gastritis; IM, intestinal metaplasia; DYS, dysplasia. [a]$P<0.05$, [b]$P<0.01$, mild IM *vs* severe IM; mild CAG *vs.* severe CAG.

Table 2 The detected positive rates of *H. pylori* for CG patients in relation to gender and age

H. pylori	Males	Females	70-77 years old	60-69 years old	50-59 years old	42-49 years old
Number	103	106	16	47	66	80
Positive (%)	64 (62.1%)	76 (71.7%)	10 (62.5%)	32 (68.1%)	39 (59.1%)	59 (73.8%)
Negative (%)	39 (37.9%)	30 (28.3%)	6 (37.5%)	15 (31.9%)	27 (40.9%)	21 (26.2%)

Table 3 Comparison of serum anti-*H. pylori* IgG and IgA levels (the optical density value) at different stages of CG

	Mild CAG		Severe CAG		Mild IM		Severe IM		DYS	
	H. pylori (+)	*H. pylori* (-)	*H. pylori* (+)	*H. pylori* (-)	*H. pylori* (+)	*H. pylori* (-)	*H. pylori* (+)	*H. pylori* (-)	*H. pylori* (+)	*H. pylori* (-)
IgG(1)	2.86±1.8[b]	0.38±0.27	4.48±2.14[bc]	0.53±0.27	3.86±1.87[b]	0.14±0.07	3.1±2.34[a]	0.55±0.29[d]	2.74±1.67[b]	0.23±0.17
IgG(2)	3.26±2.16[b]	0.46±0.24	4.52±3.27[a]	0.54±0.29	4.44±2.56[b]	0.3±0.18	3.5±1.98[b]	0.54±0.29	3.33±1.87[b]	0.72±0.97
IgA(1)	0.85±0.7[b]	0.24±0.22	0.99±0.49[b]	0.21±0.16	0.83±0.39[b]	0.1±0.06	0.93±0.79	0.33±0.16[d]	0.94±0.89[b]	0.13±0.13
IgA(2)	0.96±0.83[b]	0.25±0.21	1.08±0.59[a]	0.27±0.16	0.99±0.72[a]	0.12±0.07	0.89±0.63[a]	0.32±0.19[d]	0.78±0.6[b]	0.15±0.13

CAG, chronic atrophic gastritis; IM, intestinal metaplasia; DYS, dysplasia. (1) The first detection. (2) The second detection. [a]$P<0.01$, [b]$P<0.001$, *H. pylori* (+) *vs. H. pylori* (-). [c]$P<0.05$, *H. pylori* (+) mild CAG *vs. H. pylori* (+) severe CAG; [d]$P<0.05$, *H. pylori* (-) mild IM *vs. H. pylori* (-) severe IM.

Table 4 Diagnostic accuracy of *H. pylori* infection determined by different assemblages of symptoms

Attributes	Assemblages of symptoms	Accuracy (%)	
		H. pylori (+)	*H. pylori* (-)
Pathogenic factors and general status	Smoking, alcohol, limbs, hand and foot (all[a])	70.7%	42.3%
Pathogenic factors, general status and tongue	Smoking, alcohol, dietary regularity, limbs, and tongue quality (all[a])	74.3%	39.1%
Pathogenic factors and digestive symptoms	Smoking, alcohol, limbs, hand and foot, distending fullness in the stomach, stomachache, distending fullness in the abdomen, and nausea (all[a])	51.4%	52.2%
Digestive symptoms	Appetite, distending fullness in the stomach, stomachache, distending fullness in the abdomen, pain in the hypochondrium, pain in the abdomen, singultus, nausea, vomit, acid regurgitation and epigastric upset, heartburn, and stool (all[a])	71.4%	26.1%
Digestive symptoms and tongue	Distending fullness in the stomach, stomachache, pain in the abdomen, nausea, and fur of tongue (all[a])	72.1%	26.1%
General status	Taste, swollen, head, limbs, chest, hand and foot, spirit and sleep, emotion, complexion (all[a])	50.7%	40.6%
Total symptoms	35 items[b]	65.7 %	40.6 %

[a]P<0.05-0.001, the difference between two classes tested by the ANOVA analysis. [b]P<0.05-0.001, except dietary bias, dietary regularity, urine, oropharynx, eye, ear, physique, tongue quality, and tongue body.

Table 4 shows the diagnostic accuracy of *H. pylori* infection determined by different assemblages of clinical presentations. The overall diagnostic accuracy based on 35 presentations was 65.7 %. It could be improved by 5.7 % when the only digestive symptoms were engaged, or by 8.6 % when further information were referred such as the assemblage of pathogenic factors (smoking, alcohol), general status (tired limbs), and tongue observation according to the traditional Chinese medicine (TCM) method. However, the diagnostic accuracy could be decreased by the improper assemblages such as the general symptoms only, the pathogenic factors accompanied with some common digestive symptoms (distending fullness in the stomach, stomachache, distending fullness in the abdomen, and nausea) which had no special significance for the positive or negative *H. pylori*.

DISCUSSION

An increasing number of studies support a close relationship between *H. pylori* infection and CG, as previous described. However, the correlation between *H. pylori* infection, serum antibody and clinical symptoms at different stages of CG has not been well investigated so far. The chi-square test results in Table 1 show that the detected positive rates of *H. pylori* increase significantly in patients with mild CAG (51.3 %, 39/76), DYS and mild IM (67.7 %, 21/31), severe CAG (68.2 %, 15/22), and mild IM (72.7 %, 16/22). They reach the highest value in patients with severe IM (84.5 %, 49/58) (P<0.01). However, *H. pylori* infection exhibited no remarkable association with patient' s gender (62.1 % in males; 71.7 % in females) and age (r=0.08, P=0.241).

It has been reported that the adherence of *H. pylori* may play an important role in the pathogenesis of severe histological changes in CAG[10], IM[11] and DYS[12]. Our investigation further explored the statistic probability of *H. pylori* infection at different stages of CG. Based on these, we suggest that *H. pylori* infection may be the key factor in accelerating the occurrence and development of CG, since *H. pylori* is a pathogenic bacterium that can adhere to gastric mucosa until the atrophy and intestinal metaplasia occurring in the course of CG.

A mostly pathological mechanism of *H. pylori* infection is the immunopathological response of host. When CG occurs, a detectable specific humoral immunological response will be established. The appearance of serum antibodies such as IgG and IgA may indicate an extensive immunoreaction causing by *H. pylori* infection. The serum anti-*H. pylori* -IgG antibody, therefore, acts as a highly accurate, simple and noninvasive method in monitoring the status of *H. pylori* infection[5, 13]. In our study, both serum anti-*H. pylori* IgG and IgA are remarkably higher in *H. pylori* positive patients than those negative for *H. pylori* (P<0.001-0.01). On the one hand, in the presence of *H. pylori* infection the IgG level in severe CAG increased significantly, compared to those in mild CAG (P<0.01). These imply that the serum anti-*H. pylori* IgG and IgA are appropriate indicators to evaluate the status of *H. pylori* infection in the process of CG. On the other hand, in absence of *H. pylori* infection both serum IgG and IgA are significantly greater in severe IM than those in mild IM (P<0.01-0.05), whereas no significant difference (P>0.05) was observed between mild and severe IM in the presence of *H. pylori* infection. The increase of antibody may be due to the reminiscence of *H. pylori* infection, resulting in a certain arrearage between serum antibody and *H. pylori*. It is reported that the IgA response of gastric mucoa in CG patients can be detected even in the quiescent period of negative *H. pylori* infection due to the recent exposure to the bacterial antigens[6].

The clinical situations of CG patients are intricate. They are divergent by the pathological changes of gastric mucosa, and are affected by environmental factors[14, 15]. By means of the cluster analysis method, we found that the proper assemblages such as digestive symptoms, pathogenic factors (smoking, alcohol), general status (tired limbs), and the tongue could improve the diagnostic accuracy of *H. pylori* infection. However, the improper assemblages, such as the general symptoms only, or the pathogenic factors accompanied with some common digestive symptoms decreased the diagnostic accuracy. A preferable symptomatic assemblage has been proposed in the present study. Based on this, the diagnostic accuracies of up to 74.3 % and 40.5 %, respectively, for positivity and negativity of *H. pylori* infection have been obtained. It has been demonstrated clinically that smoking, alcohol[16] and the tongue change[17] are pathogenic factors for the *H. pylori* infection. Our study indicates that the symptomatic assemblages rather than an individual factor are closely related to the clinic significance of *H. pylori* infection. Furthermore, it has been known that the validity of CG treatment in traditional Chinese medicine (TCM) is based on the differentiation of symptom-complexes[18, 19]. Our investigation suggests that *H. pylori*-related symptomatic assemblages can be taken into consideration in practicality and methodology during the diagnoses and treatment of CG. More effective symptomatic assemblages and their effects

on *H. pylori* infection are still required.

In conclusion, the early genesis and further progression of CG are associated with *H. pylori* infection, which can be characterized by the increase of serum anti-*H. pylori* IgG and IgA with a certain arrearage. Due to the intricate clinical situation of CG, effective symptomatic assemblages are required in diagnosing *H. pylori* infection.

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