

# Immunoblot Assay in Determination of Serum Antibody Profile of Helicobacter Pylori Infection

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## Abstract

A high prevalence of antibody to cag A has been observed in H. pylori seropositive patients (HP<sup>+</sup>) living in developed countries. This is felt to be correlated with the development of serious sequelae of this infection. We examined the sero-prevalence of antibody to cag A and other specific H. pylori antigens in symptomatic and asymptomatic individuals living in Kashan.

Sera from 37 patients whose HP status was determined by culture, histology, RUT, and ELISA were enrolled in this study. The Helico – blot 2.1 western blot system was used for the detection of antibodies to specific antigens of H.pylori in sera obtained from both positive and negative HP.

Out of 85 patients, 47 (55.3%) were HP<sup>+</sup> and 38 (44.7%) HP<sup>-</sup>. Among the HP<sup>+</sup> group, 37 were tested with immunoblot system. Patients and their respective clinical disease were composed of duodenitis 19, non-ulcer dyspepsia 4, dyspepsia 4, gastritis 24, cancer and metaplasia 11 and 2 normal biopsies. In that order the corresponding antibodies to cag A was 73%,75%,75%,83%,81.8% and 50%.

The results of this study suggest that cag A antibody is more frequent in the acute status; however, this factor is diagnostically insufficient in some disease processes. In addition, cag A<sup>+</sup> phenotype can not be used as a single marker for high risk patients.

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## Introduction

**H**elicobacter pylori afflicts more than 50% of the world's population. Yet only a small percentage of subjects develop peptic ulcer disease, gastric cancer or MALT lymphoma.<sup>1</sup> It is believed that the development of this disease strongly correlates with the expression of antigenic components, specially cag A and vac A.<sup>2,3</sup>

However, the anti-HP antibody patterns have been reported to show a high degree of polymorphism that could be related to the pathological status and thus may serve as a biological predictor of the type of disease.<sup>4</sup> Cag A is expressed in approximately 60% of isolates and is highly immunogenic.<sup>5</sup> Using ELISA, an increased prevalence of antibody against cag A has been reported in both peptic ulcer and

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**Table 1:** Frequencies of 7 antibodies to H. Pylori in 44 human sera and their antibodies to predict H. Pylori infection

Immunoblot Bands(KDa)	No (%) of reacting sera from		Performance* Index (%)
	HP+ patients (n=37)	HP- patients(n=7)	
116	30 (81)	4 (57.1)	77
97	24 (64.8)	2 (28.5)	65
89	22 (59.4)	3 (42.8)	59
58	27 (73)	3 (42.8)	70
57	20 (54)	2 (28.5)	56
45	13(35.1)	1(14.2)	43
37	12 (32.4)	1 (14.2)	40

gastric cancer patients.<sup>1</sup> A positive correlation has been demonstrated between ELISA and Western Blot methods used to detect the anti-cag A antibodies.<sup>5</sup> We studied the frequencies of antibodies to cag A and other major antigens of H. pylori in symptomatic and asymptomatic individuals in Kashan and compared with those of previous reports.

### Patients and Methods

**Patients:** A total of 85 consecutive patients (57.5% male and 42.5% female with a mean age of 46.3 years) were examined at the University Hospital of Kashan, during 2000 and 2001.

The patients underwent endoscopy with multiple antral biopsies. The biopsies were processed for culture and the plates were incubated at 37 °C under microaerophilic conditions for 5-7 days, and followed by Giemsa staining. One to two specimens from each patient were checked by rapid urease test (RUT). Sera were collected and frozen at -20 °C and antibody levels were determined by ELISA.

**Immunoblot assay:** We used the Helico-blot 2.1 system (Genelabs Diagnostics, Singapore). This kit is known to contain several antigens of H. pylori including cag A, vac A and urease subunits.

### Results

Colonized patients were defined as HP<sup>+</sup> when the culture was positive by Giemsa staining, RUT and Elisa tests. Of 85 patients, 47 (55.3%) were HP<sup>+</sup> and 38 (44.7%) were HP<sup>-</sup>. Sera from the first group were tested with immunoblot system whereby 11 had metaplasia and cancer, 2 had normal biopsy, 19 had chronic gastritis and 5 had acute gastritis on pathologic examination. In endoscopy, 19 had duodenitis, 4 had nonulcer dyspepsia (NUD), 4 had dyspepsia and the remainder were unclear.

Among 38 HP<sup>-</sup> patients, 8 were tested with immunoblotting and IgG antibodies to H.pylori anti-

gens. Antibodies of the IgG class were detected to 116 (cag A), 100, 97, 89 (vac A), 84, 78, 66 (urease B), 61, 58 (HSP), 57, 45, 37, 35, 30 (urease A), 24 and 19.5 kDa Antigens.

In cases with duodenitis, 94.7% and 73.6% had antibodies to cag A and vac A, respectively. Antibody to cagA was detected in 75%, 75%, 81.8%, 83% and 50% in NUD, dyspepsia, gastritis, cancer and metaplasia and normal biopsies, respectively. In gastritis, antibodies to 66, 61, 58 KDa antigens were observed more frequently and antibodies to 35, 37 and 45 KDa bands were observed less prevalent in normal biopsy. No antibody to 45 and 35 kDa bands was demonstrated.

Among cases with cancer and metaplasia, antibodies to 116, 89, 66, 30 and 19.5 KDa bands occurred more frequently, but antibodies to 45, 37 and 35 KDa were less affected. Among patients with acute and chronic gastritis, 100% and 79%, respectively had antibody to cag A. We compared the frequencies of all antibodies in our patient population and were unable to see any correlation between those with endoscopic and histological examinations. It seems that cag A and a combination of 35 and 45 bands are able to predict the presence of acute gastritis and abnormal biopsy respectively. Table 1 demonstrates frequencies of the most important antigens between HP<sup>+</sup> and HP<sup>-</sup> patients.

In these patients, the best performance Indexes were obtained with 116 and 58 KDa bands, while none of the bands were significantly different in HP<sup>+</sup> and HP<sup>-</sup> patients (Chi-square test:  $p > 0.05$ ).

### Discussion

In this study, we determined the responses of different patients to HP antigens. These patients had various endoscopic and pathologic statuses. We found that antibodies to cag A and vac A antigens were present in 94.7% and 73.6% of duodenal ulcer patients, respectively. Antibody against cag A anti-

gen was more frequently detected in patients with cancer, metaplasia, duodenal ulcer and gastritis compared to others, though the difference was not statistically significant.

Some studies have suggested that the severity of the disease may be related to the expression by some strains of HP with respect to cag A or other virulence factors.<sup>2,6</sup> Our findings are in agreement with previous reports from China, Korea, Japan and Belgium.<sup>7-9</sup> In these countries, cag A<sup>+</sup> strains of HP are common, thus antibody against cag A is not predictive of development of more serious gastroduodenal disease<sup>10</sup> but low prevalence was reported to be present in Marrocans (30%) It has also been shown that geographic differences affect the pathogenicity of cag A<sup>+</sup> strains of HP<sup>1</sup>

In our study 81% H.pylori strains were cag A<sup>+</sup> and 19% were cag A<sup>-</sup>. Thus, among the Iranian, antibody against cag A is not predictive of the development of serious gastroduodenal disease, because of wide prevalence of cag A<sup>+</sup> strains and the possibility of insufficient factors or markers for development of peptic ulcer disease and gastric cancer in some populations. In our study, the host and environmental factors may be more important predictors of disease outcome but this correlation has remained controversial.<sup>2</sup> We compared bands between negative and positive H.pylori patients and did not find any correlation between them and preserve of the bands number. It seems that 116 (cag A) and 37 KDa bands in HP<sup>+</sup> patients are more prevalent than in HP<sup>-</sup> patients (81% vs 57.1% for cag A and 32.4% vs 14.2% for 37 KDa bands). Concordance among the immunoblot analyses findings of different authors is weak.<sup>3</sup> This may be due to both the diversity of the technical conditions and the use of different strains as the source of antigen and also the immune response or antigenic shift of infectious strains.<sup>8</sup>

In conclusion, the antigenic preparation designed for H.pylori serology must include all of H.pylori antigens. Additionally, In Iran, this test is particularly disappointing because the latent infection is highly prevalent. Thus, it seems that immunoblot and other serologic tests must be evaluated for the sensitivity and specificity. Follow-up of a cohort of H.pylori infected patients is required to confirm the value of these findings. In addition, attempts to identify further potential virulence factors of H. pylori is suggested.

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