

Original Article**Seroprevalence of anti-*Helicobacter pylori* and anti-CagA antibodies in peptic ulcer and healthy subjects in the city of Rafsanjan***Abdollah Jafarzadeh*, Mehdi Salari****Abstract**

BACKGROUND: *Helicobacter pylori* (*H. pylori*) infection is thought to play an etiologic role in several gastroduodenal diseases including gastric ulcer, duodenal ulcer, gastric MALT lymphoma, and distal gastric cancer. Several studies have suggested that *H. pylori* which express cytotoxin-associated gene A (*CagA*) may be more virulent than those that do not, but limited populations have been studied to date. The aims of the present study were to evaluate the seroprevalence of anti-*H. pylori* IgG, IgA and anti-CagA antibodies in peptic ulcer (PU) patients and healthy individuals in the city of Rafsanjan.

METHODS: A total of 60 PU patients (30 males and 30 females, aged 17 to 60 years) and 138 age-matched healthy individuals (65 males, 73 females) were enrolled in this study. Diagnosis of PU disease was established on the basis of findings by gastrointestinal endoscopy. The control group was recruited from among healthy blood donors referred to Blood Transfusion Center of Rafsanjan. A blood sample was collected from each participant and the sera were tested for the presence of anti-*H. pylori* IgG and IgA antibodies and antibody to bacterial virulence factor (*CagA*) by use of enzyme-linked immunosorbent assay. The serum concentrations of anti-*H. pylori* IgA and anti-CagA antibody were expressed as mean \pm SD in each group.

RESULTS: In PU patients the overall seroprevalence of anti-*H. pylori* IgG (95.8%), IgA (96.6%) and anti-CagA (91.6%) were higher than those observed in the control group (73.2%, $P < 0.003$; 79%, $P < 0.002$; 47.82%, $P < 0.0000001$; respectively). In the control group the prevalence of serum anti-CagA IgG antibodies was significantly higher in males compared to females (58.46% vs. 38.35%; $P < 0.01$). Moreover, the mean titer of anti-*H. pylori* IgA antibodies was significantly higher in anti-CagA+ subjects compared to anti-CagA- subjects (47.5 Uarb/ml \pm 35 vs. 27 Uarb/ml \pm 18; $P < 0.01$). Furthermore, an inverse association was found between levels and the prevalence of anti-CagA with advanced age.

CONCLUSIONS: These results show that the *H. pylori*-specific antibodies, especially anti-CagA were more prevalent among PU patients compared to the control group. Moreover, it seems that the males are more susceptible to infection with CagA+ strains compared to females. It was also found that the magnitude of the IgG response to CagA decreased with advanced age. Furthermore, the results of the present study demonstrated that the seroprevalence of *H. pylori* infection is widespread among the healthy subjects in the city of Rafsanjan.

KEY WORDS: Seroprevalence, *Helicobacter pylori*, IgG, IgA, Anti-CagA, peptic ulcer, Rafsanjan.

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H *pylori* is one of the most common chronic bacterial infections in humans. It occurs throughout the world and causes several gastroduodenal diseases including gastric ulcer, duodenal ulcer (DU), gastric MALT lymphoma, and distal gastric cancer ¹.

In 1994, the World Health Organization has categorized *H. pylori* as a group I carcinogen ².

In epidemiological studies, serum tests could offer high sensitivity and specificity. Serum assaying of anti-*H. pylori* IgG and IgA antibodies could be used to determine prevalence

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of acute and chronic infections^{3,4}. In general, the serum levels of anti-H pylori IgG antibodies were increased in the presence of infection and could be used as a marker. Serological findings of anti-H pylori IgA antibodies in symptomatic patients might have significant clinical values for the diagnosis of infection, especially if the patient is seronegative for IgG⁵.

H. pylori strains are genetically diverse. H. pylori strains may be divided into at least two subgroups based on the expression (type I) or non-expression (type II) of cytotoxin-associated gene A (CagA) and the vacuolating cytotoxin. The CagA has been identified as a possible marker of virulence of H. pylori⁶. Since the cytotoxin-associated gene product (CagA, 120 to 140 kDa) encoded by CagA is immunodominant, serum IgG antibodies to the CagA antigen may be a reliable marker of carriage of a CagA⁺ H. pylori strain^{7,8}. In Western populations, CagA⁺ H. pylori strains induce more severe gastric mucosal inflammation than CagA-negative strains and are associated with higher risks of peptic ulcer (PU) disease and gastric cancer⁹⁻¹¹. Among adults in Japan¹², China¹³, Korea¹⁴ and Singapore¹⁵, there is no clear relationship between CagA⁺ H. pylori strains and enhanced risk of disease. Moreover, it has been reported that there is wide geographical variation in the prevalence of CagA⁺ strains and their genotype^{16,17,18}.

The objective of the present study was to evaluate the seroprevalence of anti-H. pylori IgG, IgA and anti-CagA antibodies in PU patients and healthy individuals in the city of Rafsanjan.

Methods

Subjects

From March 2004 to August 2004, a cross-sectional seroprevalence study was carried out in Rafsanjan (a city that located in South-East of Iran). Sixty PU patients (mean age: 49.5 years; range: 17 to 60 years; 30 men and 30 women) and 138 healthy subjects (mean age: 45.5 years; range: 17 to 60 years; 65 men and 73 women) were included in the study.

The healthy control group was recruited among blood donors of Rafsanjan Blood Transfusion Center. The healthy controls did not undergo endoscopy and were basically healthy, with no acute or chronic illnesses. The criteria for enrolment included no history of peptic ulcer disease, no abdominal surgery, no history of therapy for H. pylori infection, and no symptoms of upper gastrointestinal disease such as indigestion, nausea, vomiting and epigastric burning pain. The PU patients had disease, confirmed by endoscopy. The selected patients had no other diseases and were not taking medications like nonsteroidal anti-inflammatory drugs. H. pylori status was assessed by biopsy-based tests (rapid biopsy urease test) and testing for the presence of serum anti-H. pylori IgG and IgA antibodies. A blood sample was obtained from each participant and the sera were separated and stored at -20°C until analysis.

Determination of H. pylori-specific antibodies in serum

The serum levels of anti-H. pylori immunoglobulin A and G were measured by using the commercial enzyme-linked immunosorbent assay (ELISA) (Equipar, Italy) according to the manufacturer's guidelines. The sensitivity and specificity of assay were >99%. Serum anti-CagA IgG antibodies levels were also assayed by ELISA method using commercial kit. The serum concentration of anti-H. pylori IgA and anti-CagA antibodies were expressed in arbitrary units per milliliter (Uarb/ml) as no International Standard is available. According to the manufacturer's guidelines, the value of 5 Uarb/ml was used to discriminate the negative samples from positive ones. Moreover, in each group the serum concentrations of anti-H. pylori IgA and anti-CagA antibody were expressed as mean \pm SD.

Statistical analysis

Differences in variables were analyzed using Mann-Whitney U-test, Chi-square and Fisher exact tests as appropriate and P values less than 0.05 were considered significant.

Results

Anti-H. pylori IgG seropositivity: The overall seroprevalence of anti-H. pylori IgG antibodies in PU patients was significantly higher than that observed in healthy subjects (95.8% vs. 73.2%; $P < 0.003$). As demonstrated in table 1, in the control group, the seropositivity of anti-H. pylori IgG was significantly higher in males as compared to females (81.5% vs. 65.8%; $P < 0.03$).

Anti-H. pylori IgA seropositivity: The overall seroprevalence of anti-H. pylori IgA antibodies was 96.6% among PU patients and 79% among healthy controls with mean titer of 57.8 Uarb/ml \pm 42.2 and 38.58 Uarb/ml \pm 31.62, respectively. The prevalence and the mean titer

of anti-H. pylori IgA were significantly higher in PU patients as compared to controls ($P < 0.002$). In PU patients and controls the seroprevalence of anti-H. pylori IgA antibodies similarly expressed in males and females. Although, in both groups the mean titer of anti-H. pylori IgA antibodies was higher in females compared to males, statistical analyses showed that the difference was not significant. However, in the control group, the mean titer of anti-H. pylori IgA was significantly higher in anti-CagA⁺ subjects compared to anti-CagA⁻ subjects (47.5 Uarb/ml \pm 35 vs. 27 Uarb/ml \pm 18; $P < 0.01$).

Table 1. Seroprevalence of anti-Helicobacter pylori and anti-CagA antibodies in peptic ulcer patients and healthy control group and according to their gender.

Group	Sex	No.	Anti-H. pylori IgG rate	Anti-H. pylori IgA rate	Anti-CagA rate	Anti-H. pylori IgA Concentration (Uarb/ml)	Anti-cagA concentration (Uarb/ml)
Healthy control	Male	65	53 (81.5%)	51 (78.5%)	38 (58.46%)	33.84 \pm 26.1*	43.14 \pm 28.6*
	Female	73	48 (65.8%)	58 (79.5%)	28 (38.35%)	42.75 \pm 35.5	54.83 \pm 29
	Total	138	101 (73.2%)	109 (79%)	66 (47.82%)	38.58 \pm 31.62	48.1 \pm 29.2
Peptic ulcer patients	Male	30	29 (96.6%)	29 (96.6%)	28 (93.3%)	48.6 \pm 35.25	41.5 \pm 34.8
	Female	30	28 (93.3%)	29 (96.6%)	27 (90%)	67.4 \pm 47.3	57.8 \pm 41.2
	Total	60	57 (95.8%)	58 (96.6%)	55 (91.6%)	57.8 \pm 42.2	51.6 \pm 37

* represent that the serum concentration of immunoglobulins expressed as mean \pm SD

Anti-CagA seropositivity: The prevalence of serum anti-CagA IgG antibodies was 91.6% in PU patients and 47.82% in healthy control group with mean titer of 51.6 \pm 37 Uarb/ml and 48.1 \pm 29.2 Uarb/ml, respectively (table 1). The prevalence of serum anti-CagA IgG antibodies was significantly higher in PU patients compared with healthy controls ($P < 0.0000001$). In the healthy control group, the prevalence of serum anti-CagA IgG antibodies was significantly higher in males compared with females (58.46% vs. 38.35%; $P < 0.01$). In contrast, the titer of serum IgG anti-CagA antibodies was markedly higher among females (54.83 \pm 29 Uarb/ml) in comparison to males (43.14 \pm 28.6 Uarb/ml) but the differences were not statistically significant ($P = 0.08$). However, in PU patients there was no significant difference be-

tween males and females regarding the prevalence of serum anti-CagA antibodies. Moreover, in the PU group, no significant difference was found between males and females regarding anti-CagA antibodies (41.5 \pm 34.8 Uarb/ml vs. 57.8 \pm 41.2 Uarb/ml; $P = 0.2$). The prevalence of serum anti-CagA antibodies was significantly ($P < 0.00001$) higher in H. pylori-infected PU patients (94.8%) compared to H. pylori-infected healthy subjects (60.5%).

Discussion

The results of the present study showed that the seroprevalence of anti-H. pylori IgG and IgA and anti-CagA antibodies was significantly higher in PU patients compared to the healthy control group. Despite significant advances in understanding the biology of H. py-

lori, the factors that determine the outcome of infection are still poorly understood. It should be explained why only a proportion of infected subjects develop peptic ulcers. The host immune response to *H. pylori* is considered to be a major factor contributing to gastric mucosal damage¹⁹. In addition to host factors, bacterial factors seem to influence the inflammatory response and the development of a more severe pathology. Bacterial virulence factors such as CagA and vacuolating cytotoxin (VacA) are associated with enhanced inflammation and cancer development. Accordingly, the fact that only 10 to 15% of those who are *H. pylori* infected develop peptic ulcers and that most remain asymptomatic throughout their lives²⁰, might be explained by differences in bacterial virulence factors, as well as differences in the host immune response.

The cytotoxin-associated protein CagA is thought to be the major *H. pylori* virulence factor involved in the pathogenesis of *H. pylori* disease is produced by only a subset of *H. pylori* isolates, defined as *H. pylori* type I. Bacterial strains which do not express CagA are known as *H. pylori* type II. Only type I *H. pylori* strains have been associated with the most-severe gastroduodenal disease in human⁶. The CagA protein of *H. pylori* is an immunodominant antigen and there is a strong correlation between the presence of serum antibodies to CagA and colonization by a CagA⁺ strain^{7,8}. Carriage of CagA⁺ strains may be determined by detection of specific-serum immunoglobulin G (IgG) antibodies to native or recombinant CagA^{7,21}. It is well demonstrated that serum testing for presence of specific IgG antibodies against the CagA is more sensitive for detection of the CagA-positive strains²². Some serological experiments have shown that antibodies to CagA correlate with the severity of the disease and that CagA-producing strains are associated with PU disease⁷.

CagA seroprevalence varies geographically¹⁷. Previous cross-sectional studies among patients who had gastroscopy in Western countries generally show a significant positive association between the presence of a CagA⁺ *H. py-*

lori strain and peptic ulcer^{7,9,23}. In some Asian countries, where CagA⁺ *H. pylori* strains predominate, such cross-sectional studies have not shown an association between CagA⁺ *H. pylori* strains and peptic ulcer¹²⁻¹⁵, except in one report²⁴. This is probably due to the observation that more than 80 percent of the non-ulcer controls in these Asian studies were CagA-positive, compared with 47% of the controls in our study. Moreover, this different ascertainment of the significance of CagA positivity may be partly attributed to differences in the populations studied. In Japan, high CagA seropositivity rates have been observed in asymptomatic adults, reinforcing the insufficiency of carriage of a CagA⁺ strain for gastric pathology¹². Among adults in Japan, there is no clear relationship between CagA⁺ *H. pylori* strains and enhanced risk of disease¹². It is possible that some Asian CagA⁺ *H. pylori* strains do not induce the accentuated tissue damage caused by Western CagA⁺ strains. Furthermore, allelic differences within CagA that distinguish Western and East Asian CagA⁺ *H. pylori* strains have been reported^{25,26}. Our results showed that more than 90% of patients with PU had antibodies to CagA. It seems that both host and bacterial factors should be considered in order to understand the pathogenesis of *H. pylori*-associated diseases. Although CagA⁺ strains may play a role in the development of peptic ulcer, they are not sufficient for this process.

It has been shown that CagA represents a bacterial virulence factor triggering the release of a concerted set of cytokines for the initiation of proinflammatory and Th1-biased immunity²⁷. *H. pylori* strains that are CagA⁺ also are associated with more-substantial gastric tissue involvement with increased neutrophil infiltration²⁸. Similarly, in gastric mucosa from persons colonized with CagA⁺ strains, increased secretion of IL-8, IL-12, IL-18 and IFN- γ secretion by gastric epithelial cell has been observed^{19,27,29}. However, various genetic host factors have also been suggested to be of importance for the development of DUs³⁰.

The results of the present study showed that more than 90% of patients with PU disease had antibodies to CagA, therefore anti-CagA antibodies seem correlate better with PU disease than anti-*H. pylori* IgG and IgA antibodies.

Our results for the first time showed that in control group the prevalence of anti-CagA was significantly higher in males compared to females. Based on these findings it seems that male gender is more susceptible to infection and colonization by CagA⁺ type I *H. pylori* strains. Our results were consistent with other studies in reporting that males are at a greater risk of *H. pylori* clinical manifestations^{31,32}. We have demonstrated the titer of serum anti-CagA antibodies to be markedly higher in females compared to males. These results represent that females may produce a protective antibody responses to CagA that can neutralize this bacterial virulence factor. These may account for higher prevalence of duodenal ulcer and gastric cancer in males. Furthermore, the results of the present study showed the magnitude anti-*H. pylori* IgA response was significantly higher in females compared to males. The reason for these differences is not known. Watanabe et al.³³ found that patients who presented high titers of anti-*H. pylori* IgA expressed a lower degree of neutrophilic infiltration on histological examination. These results suggest that anti-*H. pylori* IgA antibodies have a protective function, even though this is insufficient to completely eliminate the organism. Accordingly, it seems that this differential immune response may be directly related to the long-term clinical outcome.

We have shown a reverse association between levels and the prevalence of anti-CagA with advanced age. Moreover, higher levels of anti-*H. pylori* IgA antibodies were found in the control group in anti-CagA⁺ subjects compared to anti-CagA⁻ subjects.

CagA-positive *H. pylori* strains seem to induce markedly higher titers of anti-*H. pylori* IgA antibodies than CagA-negative strains. The reason for these observations is not known. It is possible that CagA-positive *H. pylori* strains induce an immune response with

markedly higher levels of IgA against *H. pylori*. The results of our study may provide valuable new insights into the pathophysiology of *H. pylori* and encourage further studies in this field.

The results of the present study also showed that the overall seroprevalence of *H. pylori* infection was 79% in healthy subjects in the city of Rafsanjan. Previous studies had reported that the seroprevalence of *H. pylori* infection varied from 2.8% to 60% in children^{34,35}, from 25 to 92% in adults^{36,37}, and from 13% to 90% in asymptomatic subjects^{38,39}. Different results have been reported in these studies. *H. pylori* infection varies highly between countries and often within a country as well. This discrepancy may be attributed largely to differences in age, gender, race and ethnic background, serological methods used, geographic variations, socio-economic status, job, and level of public health and education. In the present study the estimated prevalence rate was similar to those obtained in other studies from developing countries. Previous Iranian studies also showed a seroprevalence of about 50% in asymptomatic subjects⁴⁰, which was lower than the 79% found in the present study.

In conclusion, the results of this study show that the anti-*H. pylori* IgG, IgA and anti-CagA antibodies (especially anti-CagA) were more prevalent among PU patients compared to the healthy control group. These findings confirm that carriage of CagA⁺ strains plays an important role in PU pathogenesis. Thus, not all *H. pylori* strains are same in their relation to disease and physicians should consider *H. pylori* strain characteristics, such as CagA status, while attempting to optimize care for their patients. Based on the differential prevalence of anti-CagA antibodies, it seems that the male gender is more susceptible to infection with CagA⁺ strains compared to females. However, a reverse association was found between the levels and the prevalence of anti-CagA, and increased age. Furthermore, these results demonstrated that the *H. pylori* infection is widespread among healthy subjects in the city of Rafsanjan.

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