Helicobacter pylori anti-CagA antibodies: Prevalence in symptomatic and asymptomatic subjects in Turkey

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BACKGROUND: Several reports have shown the prevalence of anti-CagA antibodies to be associated with the development of peptic ulcer diseases, while others have indicated that there is no such association.

AIM: To examine the prevalence of antibodies to CagA and other Helicobacter pylori antigens in symptomatic and asymptomatic subjects in Turkey.

SUBJECTS AND METHODS: Sixty-six symptomatic subjects, 16 to 74 years of age, were examined for H pylori by biopsy-based tests and ELISA. One hundred nineteen asymptomatic subjects, 20 to 65 years of age, were also tested serologically for the presence of H pylori. Samples from both groups that were found to be positive for H pylori by ELISA were then tested by immunoblotting.

RESULTS: Fifty-four (82%) symptomatic subjects and 76 (64%) asymptomatic subjects were found to be H pylori-positive by ELISA. Samples from 30 symptomatic subjects who were found to be H pylori-positive by ELISA were analyzed by immunoblotting. Antibodies to CagA (116 kDa) antigen were detected in immunoblots of 11 of 14 (79%) with chronic gastritis, 12 of 13 (92%) with duodenal ulcer and three of three (100%) with gastric cancer. Antigens of the following molecular weights were also detected in these 30 subjects: 89 kDa (VacA) in 21 (70%), 37 kDa in 21 (70%), 35 kDa in 19 (63%), 30 kDa in 27 (90%) and 19.5 kDa in 19 (63%). Immunoblots of 40 ELISA-positive asymptomatic subjects showed that 33 (83%) had antibodies to CagA antigen, 26 (65%) to VacA antigen, 30 (75%) to a 37 kDa antigen, 30 (75%) to a 35 kDa antigen, 39 (98%) to a 30 kDa antigen and 36 (90%) to a 19.5 kDa antigen.

CONCLUSIONS: Antibodies to CagA antigen were prevalent in both groups, regardless of the presence of gastroduodenal disease.

Key Words: CagA; Helicobacter pylori; Immunoblot; Peptic ulcer disease

Résumé à la page suivante


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Anticorps anti-CagA d’Helicobacter pylori : prévalence chez des sujets turcs symptomatiques et asymptomatiques

CONTEXTE : Plusieurs rapports ont fait état de la prévalence des anticorps anti-CagA associés au développement d’ulcères gastro-duodénaux, alors que d’autres nient l’existence d’un tel lien.

BUT : Étudier la prévalence des anticorps anti-CagA et de ceux dirigés contre d’autres antigènes d’Helicobacter pylori chez des sujets turcs symptomatiques et asymptomatiques.

SUJETS ET MÉTHODE : Soixante-six sujets symptomatiques âgés de 16 à 74 ans ont été soumis à des tests de dépistage de H. pylori au moyen du test ELISA et de biopsies. Cent dix-neuf sujets asymptomatiques de 20 à 65 ans ont aussi subi des tests sérologiques et histologiques. Sixty-six patients attending the endoscopy unit at the Samatya Hospital, Istanbul, Turkey, from March to July 2000, with dyspepsia and abdominal pain as their main complaint, were selected randomly. The study group comprised 38 men and 28 women, 16 to 74 years of age (average age 45 years). All patients underwent endoscopy, and three biopsies were taken from the antral part of the stomach for CLO, culture and histology. A serum sample was obtained from each subject and stored at –20°C. A questionnaire was filled out for each subject.

RÉSULTATS : Cinquante-quatre (82 %) patients symptomatiques et 76 (64 %) patients asymptomatiques se sont révélés H. pylori-positifs selon le test ELISA. Les spécimens de 30 sujets symptomatiques qui se sont révélés H. pylori-positifs ont été soumis au test immunoblot. Des anticorps anti-antigène CagA (116 kDa) ont été détectés dans les immunoblots de 11 sujets sur 14 (79 %) souffrant de gastrite chronique, de 12 sujets sur 13 (92 %) souffrant d’ulcère duodénal et des trois (100 %) sujets atteints d’un cancer de l’estomac. Les antigènes ayant les masses moléculaires suivantes ont aussi été détectés chez ces 30 sujets : 89 kDa (VacA) chez 21 (70 %), 37 kDa chez 21 (70 %), 35 kDa chez 19 (63 %), 30 kDa chez 27 (90 %) et 19,5 kDa chez 19 (63 %). Les immunoblots de 40 sujets asymptomatiques H. pylori-positifs selon le test ELISA ont montré que 33 (83 %) présentaient des anticorps anti-antigène CagA, 26 (65 %) des anticorps anti-VacA, 30 (75 %) des anticorps dirigés contre un antigène de 37 kDa, 30 (75 %) des anticorps dirigés contre un antigène de 35 kDa, 39 (98 %) des anticorps dirigés contre un antigène de 30 kDa et 36 (90 %) des anticorps dirigés contre un antigène de 19,5 kDa.

CONCLUSIONS : Les anticorps dirigés contre l’antigène CagA étaient prévalents dans les deux groupes, peu importe la présence de maladie gastro-duodénale.

Helicobacter pylori colonize the stomachs of approximately 50% of the world’s population (1,2). The prevalence of infection ranges from 25% in developed countries to more than 90% in developing countries (2-5). In spite of this high prevalence, only a small percentage of infected individuals develop peptic ulcer disease (2), of which the prevalence of CagA antibodies and duodenal ulcer disease ranges from 25% in developed countries to more than 90% in developing countries (2-5). In spite of this high prevalence, only a small percentage of infected individuals develop peptic ulcer disease (2), of which the prevalence of CagA antibodies and duodenal ulcer disease ranges from 25% in developed countries to more than 90% in developing countries (2-5).

Several tests have been used to diagnose H. pylori infection; these are divided into biopsy-based tests (CLO, culture, histology, polymerase chain reaction) and nonbiopsy-based tests (serology, urea breath test) (20). Serological tests are noninvasive and are used to detect immunoglobulin (Ig) G, IgA and IgM antibodies in the sera of H. pylori-infected subjects. The ELISA test is the most widely used test for the detection of such antibodies in epidemiological studies (4,21,22). Immunoblot enables the detection of antibodies against specified H. pylori antigens, such as CagA and VacA antigens (21,23). The aim of the present study was to detect H. pylori antibodies in symptomatic and asymptomatic subjects by the ELISA test, and to determine the prevalence of anti-CagA antibodies and other H. pylori-specific antigens in both groups by immunoblotting.

Subjects and Methods

Subjects

Symptomatic subjects: Sixty-six patients attending the endoscopy unit at the Samatya Hospital, Istanbul, Turkey, from March to July 2000, with dyspepsia and abdominal pain as their main complaint, were selected randomly. The study group comprised 38 men and 28 women, 16 to 74 years of age (average age 45 years). All patients underwent endoscopy, and three biopsies were taken from the antral part of the stomach for CLO, culture and histology. A serum sample was obtained from each patient and stored at –20°C.

Asymptomatic subjects: One hundred nineteen subjects (blood donors, school teachers and health workers), 20 to 65 years of age (average age 37 years), with no history of abdominal pain were selected randomly. A serum sample was obtained from each subject and stored at –20°C. A questionnaire was filled out for each subject.

CLO test

One biopsy was placed into the CLOtest (Clia Waived, USA) and kept at room temperature. The results were recorded as specified by the manufacturer.

Culture

Biopsies for culture were dissected into small pieces using a sterile blade and then inoculated onto Colombia agar medium (Oxoid, United Kingdom), with 5% defibrinated sheep blood and selective supplements (H pylori-selective supplement, Oxoid). The cultures were incubated in anaerobic jars, in humidified, microaerophilic conditions, using CompyGen Gas pack (Oxoid) at 37°C for five days. The organisms were identified as H. pylori by Gram staining, colony morphology, and urease, oxidase and catalase-positive reactions.
**Histology**

Antral biopsies placed in 10% formalin were sent to the histopathology laboratory. Sections were stained with hematoxylin and eosin stain, and modified Giemsa stain, and examined by an experienced histopathologist.

**Enzyme immunoassay**

The ELISA-kit SIA-\textit{H pylori} (Sigma, USA) was used to detect IgG antibodies to \textit{H pylori} in human sera. The test was conducted according to the manufacturer's instructions. Samples were washed with the EL×50 Auto Strip Washer, and the results were read with the EL×800 Universal Microplate Reader (Bio-Tek Inc, USA). All samples were tested at the same time.

**Immunoblot assay**

The Helico-Blot 2.1 (Genelabs Diagnostics, Singapore) was used to test the ELISA-positive serum samples from both groups. The test is based on Western blot assay of \textit{H pylori} whole-cell antigens that is made from bacterial lysate of a strain of \textit{H pylori} that is known to cause ulcers. Strips were marked at the 116 (CagA), 89 (VacA), 37, 35, 30 and 19.5 kDa antigen lines and at an additional antigen line, designated the current infection marker (CIM). The recommended criteria for determining \textit{H pylori} seropositivity as specified by the manufacturer is any one of:

- the detection of 116 kDa (CagA) band with one or more bands of 89, 37, 35, 30 (UreA) and 19.5 kDa together, or with CIM;
- any one band of the 89, 37 or 35 kDa, with or without the CIM; or
- detection of both the 30 and 19.5 kDa bands, with or without the CIM.

Imaging and analysis of immunoblots were done using Gel Doc 2000 and Quantity One quantification software (Bio-Rad, USA).

**Statistical analysis**

Data were analyzed using SPSS 10.1 (SPSS Inc, USA). P<0.05 was considered to be statistically significant.

### RESULTS

**Symptomatic subjects**

Biopsies were taken, and the presence of \textit{H pylori} was determined through CLO, culture and histology. Patients were considered to be infected if one test was positive. Fifty-seven of 66 patients were found, by either one of the biopsy-based tests, to be infected with \textit{H pylori}. Results of the ELISA test were compared with those of the biopsy-based tests. ELISA was shown to have 89% sensitivity (95% CI 81.5 to 95.5%), 100% specificity, 100% positive predictive value and 67% negative predictive value (95% CI 55.5 to 78.3%). Among the 66 symptomatic subjects included in the study, 32 of 38 (84%) men and 22 of 28 (79%) women were found to be \textit{H pylori}-positive by ELISA – a total of 54 of 66 (82%) subjects.

Immunoblot assay was performed on 30 randomly selected ELISA-positive sera from symptomatic subjects. Table 1 shows the correlation between the prevalence of CagA antibodies and age in symptomatic subjects who were found to be \textit{H pylori}-positive by biopsy-based tests. Statistical analysis showed no significant difference between these age groups. CagA antibodies were detected in patients with the following histological findings: 11 of 14 (79%) with chronic gastritis, 12 of 13 (92%) with duodenal ulcer and three of three (100%) with gastric cancer (one patient also had gastric ulcer) (Table 2). VacA antibodies

<table>
<thead>
<tr>
<th>Molecular weight of antigens (kDa)</th>
<th>116 (CagA)</th>
<th>89 (VacA)</th>
<th>37</th>
<th>35</th>
<th>30</th>
<th>19.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastritis, n=14 (%)</td>
<td>11 (79)</td>
<td>8 (57)</td>
<td>9 (64)</td>
<td>9 (64)</td>
<td>11 (79)</td>
<td>7 (50)</td>
</tr>
<tr>
<td>Duodenal ulcer, n=13 (%)</td>
<td>12 (92)</td>
<td>11 (85)</td>
<td>10 (77)</td>
<td>8 (62)</td>
<td>13 (100)</td>
<td>10 (77)</td>
</tr>
<tr>
<td>Gastric cancer, n=3 (%)</td>
<td>3 (100)</td>
<td>2 (67)</td>
<td>2 (67)</td>
<td>2 (67)</td>
<td>3 (100)</td>
<td>2 (67)</td>
</tr>
</tbody>
</table>

**TABLE 1**

Correlation between the presence of \textit{Helicobacter pylori} anti-CagA antibodies, detected by immunoblotting, and age in symptomatic subjects who were found to be \textit{H pylori}-positive by biopsy-based tests

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>CagA+ subjects, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;45 (n=14)</td>
<td>12 (86)</td>
</tr>
<tr>
<td>&lt;45 (n=16)</td>
<td>12 (75)</td>
</tr>
<tr>
<td>Total (n=30)</td>
<td>24 (80)</td>
</tr>
</tbody>
</table>

P>0.05

**TABLE 2**

Prevalence of antibodies to \textit{Helicobacter pylori} antigens in immunoblots of symptomatic subjects with peptic ulcer diseases
were detected in eight of 14 (57%) subjects with chronic gastritis, 11 of 13 (85%) with duodenal ulcer and two of three (67%) with gastric cancer. Antibodies to the other four antigens varied also in their frequency pattern (Table 2). The CIM appeared in 25 of 30 (83%) positive strips.

Asymptomatic subjects

Among the 119 asymptomatic subjects, 76 (64%) were found to be *H pylori* positive by ELISA. The sera from 40 of these subjects (randomly selected) were analyzed by immunoblotting. Table 3 compares the immunoblot results of *H pylori* antigens in symptomatic and asymptomatic subjects. Immunoblots revealed antibodies to CagA antigen in 26 (87%) symptomatic and 33 (83%) asymptomatic subjects. The frequencies of immunoreactive bands to VacA and to other antigens in the immunoblots of both groups were variable. The CIM was detected in 35 of 40 (87%) positive strips. Figure 1 shows representative immunoblot strips from *H pylori*-positive, asymptomatic subjects.

TABLE 3
Prevalence of antibodies to *Helicobacter pylori* antigens in immunoblots of symptomatic and asymptomatic subjects

<table>
<thead>
<tr>
<th>Molecular weight of antigens (kDa)</th>
<th>Subject group</th>
</tr>
</thead>
<tbody>
<tr>
<td>116 (CagA)</td>
<td>89 (VacA)</td>
</tr>
<tr>
<td>Symptomatic, n=30 (%)</td>
<td>21 (70)</td>
</tr>
<tr>
<td>Asymptomatic, n=40 (%)</td>
<td>21 (70)</td>
</tr>
<tr>
<td></td>
<td>19 (63)</td>
</tr>
<tr>
<td></td>
<td>27 (90)</td>
</tr>
<tr>
<td></td>
<td>19 (63)</td>
</tr>
</tbody>
</table>

Asymptomatic subjects

Among the 119 asymptomatic subjects, 76 (64%) were found to be *H pylori* positive by ELISA. The sera from 40 of these subjects (randomly selected) were analyzed by immunoblotting. Table 3 compares the immunoblot results of *H pylori* antigens in symptomatic and asymptomatic subjects. Immunoblots revealed antibodies to CagA antigen in 26 (87%) symptomatic and 33 (83%) asymptomatic subjects. The frequencies of immunoreactive bands to VacA and to other antigens in the immunoblots of both groups were variable. The CIM was detected in 35 of 40 (87%) positive strips. Figure 1 shows representative immunoblot strips from *H pylori*-positive, asymptomatic subjects.

DISCUSSION

In this study, the prevalence of *H pylori* in gastric biopsies of symptomatic subjects was determined by CLO, culture and histology. If one test was positive, patients were considered to be infected. Others have reported using a similar approach (18). In comparison with the biopsy-based tests, serology proved to be useful for the detection of *H pylori* infection (24). A review of the overall performance of the commercially available serology kits that measure IgG antibodies showed that serology is an accurate method of diagnosing *H pylori* in patients (25). Comparison between serology and the combination of CLO, histology and culture revealed 94% sensitivity and 88% specificity (18). Compared with the biopsy-based tests, ELISA was 89% sensitive and 100% specific in detecting *H pylori* infection in symptomatic subjects. These results were in agreement with those of an earlier study (25).

*H pylori* is associated with several gastroduodenal diseases such as gastritis, gastric ulcer, duodenal ulcer, gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma (2,26,27). CagA has been associated with the development of peptic ulcer disease and gastric cancer. It is expressed in approximately 60% of the isolates and the protein is highly immunogenic (4), while vacA gene, although present in nearly all strains, is expressed in only 50% of the isolates (18).

Immunoblotting is a more sensitive serological test than ELISA for the diagnosis of *H pylori* infection and the detection of specified antigens (6,15,19). With immunoblot testing, no significant age difference in the response to CagA antigen between patients older than 45 years and those younger than 45 years of age was found, indicating that the prevalence of CagA-positive strains is not restricted to...
older age and that subjects in different age groups mount a good antibody response to the CagA antigen.

An increase in the prevalence of anti-CagA antibodies has been reported in patients with peptic ulcer disease and those with gastric cancer (2,4,20,28). There appears to be major geographic differences in the prevalence of CagA-positive strains. In Australia, the prevalence of antibodies to CagA in patients with duodenal ulcer was higher than that in those with nonulcer dyspepsia and in asymptomatic people, while in China, no significant difference in the prevalence of CagA antibodies between asymptomatic and gastric cancer groups was found (4). In another study, host and environmental factors were found to be more important in the development of the disease (29). In the present study, the detection of such antibodies in the sera of symptomatic and asymptomatic subjects indicates that CagA-positive \textit{H. pylori} strains prevailed in both groups. These results appeared to be in agreement with those of previously published reports (4,15-17,27,30). Rocha et al (23) indicated that immunoblotting using the Helico-Blot kit is useful for the diagnosis of \textit{H. pylori} infection in children and that the frequency of immunoreactive bands to CagA antigen is higher in children with duodenal ulcer than in children without the disease. However, Mitchell et al (31) reported in an earlier study that antibody to CagA is not a marker for specific disease in children. More recently, Figueiredo et al (24) compared the Helico-Blot kit with two other noncommercially developed enzyme immunoassays and concluded that the detection of anti-CagA antibodies is strongly dependent on the test used. The limitations reported by Figueiredo et al (24) in their interpretation of the results were due to several factors that included observer visual scoring, low intensity of some signals and variation between different strip batches that prevent accurate alignment. These limitations were overcome in our study by the use of Gel Doc and the Quantity One software program for imaging and analysis.

Differences in the frequencies of the immunoreactive bands to the six antigens between the symptomatic and asymptomatic subjects in the present study were attributed to individual and strain variability. In addition, the human gastric mucosa colonized by \textit{H. pylori} shows a variety of responses that may vary greatly in the intensity and distribution of the histological responses and correlation with the clinical outcome (11). The detection of CIM did not seem to add any further information to the interpretation of the results.

**CONCLUSIONS**

Antibodies to CagA antigen are prevalent in both symptomatic and asymptomatic subjects, regardless of the gastroduodenal disease. This gene product might be insufficient to act as a marker for the development of the disease in some populations.

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