Detection of *H pylori* infection by ELISA and Western blot techniques and evaluation of anti CagA seropositivity in adult Turkish dyspeptic patients

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Abstract

AIM: To detect *H pylori* infection and to evaluate the anti CagA seropositivity in adult Turkish dyspeptic patients.

METHODS: We evaluated anti-*H pylori* IgA, IgG and anti-CagA antibodies using commercial enzyme-linked immunooassay (ELISA) and Western blot in dyspeptic Turkish patients. *H pylori* status was determined by histology and rapid urease testing.

RESULTS: Fifty-six patients were entered. Forty-eight (85.7%) out of the 56 patients were positive for *H pylori*. *H pylori* IgG seropositivity was 82.1%, IgA seropositivity 48.2%. CagA ELISA showed that IgG was positive in 50% and IgA in 30.4% of those with *H pylori* infections. Western blot showed that IgG seropositivity was 80.4% and IgA seropositivity 33.9%. Western blot detected IgG antibodies with reactivity to CagA in 50%, VacA in 62.5%, UreB in 87.5%, UreA in 80.4%, and OMP in 57.1%. None of the tests had a sensitivity and specificity above 80%.

CONCLUSION: None of these commercial tests seems clinically useful for *H pylori* detection in adult dyspeptic patients, while Western blot can give seropositivity and determine anti-CagA, VacA virulence factor status of Turkish dyspeptic patients in the Izmir region.

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**Key words:** *H pylori*; Serum CagA; Enzyme-linked immunooassay; Western blot; Serodiagnosis; Dyspeptic patients

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INTRODUCTION

*H pylori* colonizes in the mucosa of the human stomach where it establishes a long-term infection associated with acute or chronic gastric inflammation, which may progress to peptic ulcer disease, atrophic gastritis with intestinal metaplasia, or gastric cancer. A variety of clinical outcomes of *H pylori* infection are associated both with host factors and with bacterial virulence factors[1]. Several *H pylori* virulence genes have been identified, of these, oipA, vacA, cagA and babA appear to play a major role in pathogenicity. The cytotoxin-associated gene (CagA) is a marker for the cag pathogenicity island (PAI), a 40-kb genomic region[1,2]. Most strains of *H pylori* from patients with peptic ulcer disease carry the CagA gene (*CagA* positive strains)[1] and the presence of the CagA gene increases the risk of developing peptic ulceration, atrophic gastritis, and adenocarcinoma in the stomach. Consequently the discrimination of *CagA* positive and *CagA* negative *H pylori* strains might prove useful in predicting the chance for complications as well as for clinical and epidemiological studies of *H pylori* infection[1,2].

*H pylori* cag PAI status is typically assessed by the immune response to the immuno-dominant CagA or by detection of the CagA gene. Available serological tools to characterize the infecting *H pylori* strains have been questioned because of their inadequate sensitivity and specificity[3].

Current guidelines for the management of *H pylori* infection recommend eradication treatment without performing endoscopy in patients (< 45 years of age) with no alarm symptoms, thus making the availability of simple and reliable noninvasive tests important[4]. Currently
available noninvasive tests for the diagnosis of \textit{H pylori} infection include UBT, stool antigen test, and detection of anti-\textit{H pylori} antibodies (e.g., serology).

This study compared five different diagnostic tests: rapid urease test, histology, anti-\textit{H pylori} CagA Enzyme-linked immunoassay (ELISA) of IgA and IgG, anti-\textit{H pylori} ELISA of IgA and IgG, Western blot of IgA and IgG including CagA and other antigens in untreated adult dyspeptic Turkish patients.

### MATERIALS AND METHODS

**Patients**

The study population consisted of adult Turkish dyspeptic patients admitted to the Dokuz Eylül University Hospital, Gastroenterology Clinic. The patients were eligible if they had no \textit{H pylori} eradication treatment in the previous 6 mo or did not receive antibiotics, H2-receptor antagonists, sucralfate or omeprazole one month prior to examination, and had no previous history of gastric or duodenal malignancies. The patients with a history of coagulopathy or other disorders that were contraindications for endoscopy and/or biopsy sampling were excluded.

**Endoscopy and biopsy sampling**

Two antrum and two corpus biopsy specimens were taken from each patient undergoing upper endoscopy: one from the antrum and one from the corpus were used for the rapid urease test and the others were immediately fixed and transported in 10\% phosphate-buffered formalin solution for histopathologic examination.

**Histopathologic examination of biopsy specimens**

Paraffin-embedded gastric biopsy specimens were routinely processed. Hematoxylin and eosin, alcian blue and Giemsa stains were used for morphologic examination of \textit{Helicobacter}-like organisms (HLO). The updated Sydney system was used for determining gastritis activity and grading bacterial density of \textit{H pylori}. Gastritis activity was graded on a four-point scale as none (grade 0), mild (grade 1), moderate (grade 2), and severe (grade 3)\cite{5}. \textit{H pylori} infection was defined as positivity of histopathology and rapid urease test. Histology was performed by a specialized pathologist. A patient was defined as \textit{H pylori} negative when both histologic examination and urease test were negative and as \textit{H pylori} positive when both histologic examination and urease test were positive.

**Serological tests and sera**

Sera were collected on the same day as the biopsies from patients undergoing endoscopy. Serum samples were aliquotted and stored at -20\(^\circ\)C until used.

**Enzyme-linked immunoassay**

Anti-\textit{H pylori} IgA and IgG Western blot, IgA and IgG ELISA, anti-CagA IgA and IgG ELISA (EUROIMMUN Medizinische Labordiagnostika, Lübeck, Germany) were used to detect the presence of \textit{H pylori}-specific serum antibodies according to the manufacturer’s instructions. The recommended cut-off values were used.

**Immunoblot assay**

Western blot (EUROIMMUN Medizinische Labordiagnostika, Lübeck, Germany) was used to detect the presence of \textit{H pylori}-specific serum antibodies according to the manufacturer’s instructions. Western blot test consisted of \textit{H pylori} antigen extracts with the following molecular weights of the corresponding bands to these proteins which were 120 kDa (CagA); 95 kDa (VacA); 67 kDa (flagellar sheet protein, nonspecific); 66 kDa (UreB); 57 kDa (heat-shock protein homolog); 33 kDa, 30 kDa, 29 kDa (UreA); 26 kDa, 19 kDa and 17 kDa. Anti-\textit{H pylori} IgG antibodies were positive determined by Western blot when the 120 kDa (CagA) band, as well as at least two distinctive antigen bands from species-specific and highly specific antigens with the molecular weights of 95 kDa (VacA), 33 kDa, 30 kDa, 29 kDa (UreA), 26 kDa, 19 kDa and 17 kDa were present. Faint bands or no band was regarded as negative.

**Statistical analysis**

The McNemar’s \(\chi^2\) test was used. The sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV), the likelihood ratios (+, -) and diagnostic accuracies of the anti-\textit{H pylori} CagA IgA and IgG ELISA, IgA and IgG ELISA were computed against the gold standards (SPSS, version 11.0 for Windows). The receiver operating characteristic (ROC) curve analysis was also done.

**Ethics**

The research protocol was approved by the Institutional Review Board and the Ethical Committee of the Dokuz Eylül University, Faculty of Medicine, Izmir, Turkey. All patients gave their written consent to participate in the study.

### RESULTS

A total of 56 adult Turkish dyspeptic patients (19 males, 37 females; mean age, 46.41 ± 13.12 years; age range, 21 to 78 years) were enrolled. \textit{H pylori} infection was diagnosed in 48 (85.7\%) patients by rapid urease test and histopathology. ELISA showed that \textit{H pylori} seropositivity was 48.2\% for IgA and 82.1\% for IgG antibodies. CagA ELISA showed that IgA seropositivity was 30.4\% and IgG seropositivity was 50\% which was the same as the Western blot results. Western blot showed that IgA was positive in 33.9\% and IgG in 80.4\%. No significant statistical difference was found between CagA IgA and IgG antibodies by CagA ELISA but anti-\textit{H pylori} IgG ELISA was significantly different with gold standards (\(P < 0.05\)) (Table 1, Table 2, Table 3).

Anti-\textit{H pylori} Western blot of IgG antibodies also showed reactivity with p120 (CagA), p95 (VacA), p66 (UreB), p29 (UreA) and p19 (OMP) antigens: 28, 35, 49, 45 and 32 were positive, respectively (Table 4). A significant correlation with gastritis activity was also observed according to updated Sydney system (\(P < 0.0001\)). ROC analyses of all serological tests used in the study were also done and did not show any difference.
**DISCUSSION**

*H. pylori* infection can be diagnosed by a variety of invasive and non-invasive tests. Serology can be performed on non-invasively collected clinical samples. Serological detection of infection with a CagA containing strain of *H. pylori* by anti-CagA ELISA and Western blot of CagA is the only noninvasive diagnostic test at present available for assessing strain virulence potential and possible disease risk. The reliability of CagA serology as a predictive test for determining the CagA genotype of the infecting strain is important because various serological assays are now available.

Infection with *H. pylori* evokes both local and systemic antibody responses. CagA is the important pathologic marker with a high immunogenic response. In Europe, CagA-positive *H. pylori* has been reported to account for 60% to 70% of *H. pylori* strains, while reports from East Asian countries have shown that more than 90% of *H. pylori* strains are CagA positive irrespective of the disease presentation.

Noninvasive tests are becoming more and more important in the clinical management of dyspeptic patients, followed by the treatment of *H pylori* in primary care according to “the Maastricht 2-2000 European Consensus report”. A “test and treat” approach is recommended in adult patients under the age of 45-55 years with no alarm symptoms. But choosing the ‘right’ noninvasive test is not easy. Serological tests have limitations in detecting the exposure to *H. pylori*. A meta-analysis of 21 studies with commercially available ELISA serology kits has reported an overall sensitivity and specificity of 85% and 79%, respectively. Thus, the accuracy of these tests is no longer adequate to justify their use on clinical or economic grounds. However, new serological kits are available to detect antibodies to CagA or VacA by ELISA and Western blot, and can achieve rather good sensitivity and specificity. Because serological tests for *H pylori* vary

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**Table 1** Detection of *H pylori* status by Western blot, ELISA and CagA ELISA of IgA

| Patients | Western blot + (%) | *H pylori*/IgA ELISA + (%) | CagA ELISA + (%) | Total |
|----------|--------------------|-----------------------------|------------------|-------|
| *H pylori* (+) (n = 48) | 17 (89.5) | 24 (80.0) | 7 (100) | 26 (96.3) | 22 (73.9) | 16 (94.1) | 32 (82.1) | 48 (85.7) |
| *H pylori* (-) (n = 8) | 2 (10.5) | 6 (20.6) | 0 | 1 (3.7) | 7 (24.1) | 1 (5.9) | 7 (17.9) | 8 (14.3) |
| Total | 19 | 30 | 7 | 27 | 29 | 17 | 39 | 56 |

**Table 2** Detection of *H pylori* status by Western blot, ELISA and CagA ELISA of IgG

| Patients | Western blot + (%) | *H pylori*/IgG ELISA + (%) | CagA ELISA + (%) | Total |
|----------|--------------------|-----------------------------|------------------|-------|
| *H pylori* (+) (n = 48) | 40 (88.9) | 1 (50.0) | 7 (77.8) | 42 (91.3) | 6 (85.7) | 26 (92.9) | 22 (78.6) | 48 (85.7) |
| *H pylori* (-) (n = 8) | 5 (11.1) | 1 (50.0) | 2 (22.2) | 4 (8.7) | 4 (57.1) | 2 (71) | 6 (21.4) | 8 (14.3) |
| Total | 45 | 2 | 9 | 46 | 10 | 28 | 28 | 56 |

**Table 3** Results of anti-*H pylori* ELISA and CagA ELISA of IgA, IgG in dyspeptic patients

| Antigens | Sensitivity | Specificity | PPV | NPV | LR+ | LR- | Diagnostic accuracy |
|----------|-------------|-------------|-----|-----|-----|-----|---------------------|
| ELISA IgA | 54.2 | 87.5 | 63.3 | 0.62 | 4.33 | 58.9 |
| ELISA IgG | 87.5 | 50 | 91.3 | 40 | 1.75 | 91.3 |
| CagA ELISA IgA | 33.3 | 87.5 | 94.1 | 0.38 | 2.67 | 41.1 |
| CagA ELISA IgG | 54.2 | 75 | 92.9 | 21.4 | 0.72 | 21.7 | 57.1 |

PPV: Positive predictive value; NPV: Negative predictive value; LR: Likelihood ratio.

**Table 4** Anti-*H pylori* IgG Western blot results of the distribution of different antigens

| Antigens | Positive patients (n = 56) | % |
|----------|-----------------------------|---|
| p120 (CagA) | 28 | 50 |
| p95 (VacA) | 35 | 62.5 |
| p75 | 18 | 32.1 |
| p67 (FSH) | 23 | 41.1 |
| p66 (UreB) | 49 | 87.5 |
| p57 (HSP homolog) | 37 | 66.1 |
| p54 (Flagellin) | 24 | 42.9 |
| p50 | 24 | 42.9 |
| p41 | 28 | 50 |
| p33 | 13 | 23.2 |
| p30 | 24 | 42.9 |
| p29 (UreA) | 45 | 80.4 |
| p26 | 23 | 41.1 |
| p19 (OMP) | 32 | 57.1 |
| p17 | 20 | 35.7 |
in different populations, largely due to the \textit{H pylori} strain heterogeneity and variations in antigenic preparations, their accuracy must be confirmed in the target populations\cite{10,12}. Unfortunately, none of these new tests has a sensitivity and specificity above 80\% and therefore they should be used with care for \textit{H pylori} detection in Turkish dyspeptic patients in our region.

Serin \textit{et al}\cite{13} compared the frequencies of serum positive CagA in patients from two separate regions of Turkey and found that the rate of CagA serum prevalence is high (97.2\%) in patients with non-ulcer dyspepsia but similar in \textit{H pylori}-positive patients. They also observed similar frequencies of CagA (+) \textit{H pylori} strains in dyspeptic patients irrespective of ulcer status, suggesting that factors other than CagA can contribute to severe gastrointestinal pathology in patients with \textit{H pylori}. We found that 50\% of patients with \textit{H pylori} infection had CagA positive serology which is below the percentage expected, suggesting that the test is not accurate.

A set of serological tests may give more accurate determinations of \textit{H pylori} infection than one test detecting specific antibody or bacterial antigen. In this study it seemed that there was a good correlation with Western blot and ELISA test results compared to the gold standard methods. \textit{H pylori} seropositivity was 82.1\% by ELISA IgG antibodies and 80.4\% by Western blot IgG antibodies. We suggest that ELISA, CagA ELISA and Western blot techniques should be used together where both the incidence of \textit{H pylori} and the treatment failure rates are very high. We should emphasize more on anti-CagA status determination because anti-CagA positive patients are substantially associated with atrophic gastritis, persistent active inflammation and atrophic gastritis.

In conclusion, anti-\textit{H pylori} IgA testing seems clinically useless and IgG testing is not clinically possible because the tests are based on antigens from European strains. Since Izmir is a cosmopolitan area under the influence of both Asian and Western countries, the common strains in different populations, largely due to the \textit{H pylori} strain heterogeneity and variations in antigenic preparations, their accuracy must be confirmed in the target populations\cite{10,12}. Unfortunately, none of these new tests has a sensitivity and specificity above 80\% and therefore they should be used with care for \textit{H pylori} detection in Turkish dyspeptic patients in our region.

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