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The effect of CagA status on response to *Helicobacter pylori* eradication therapy in Western Turkey

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Abstract

If cytotoxin-associated gene A (CagA) status affects the response rates of therapy, then it may be possible to predict *Helicobacter pylori* eradication rates. We aimed to evaluate the response to eradication treatment of *H. pylori* infection in CagA-positive and CagA-negative patients. A total of 184 patients (93 males, 91 females, mean age 42.6 ± 12.8 years) with *H. pylori*-positive chronic gastritis were studied. Subjects underwent a gastroscopy and biopsy specimens were taken from the gastric antrum, body, and fundus. Before the eradication therapy was given all patients were tested for CagA, TNF-α and gastrin levels. They were then prescribed lansoprazole (30 mg bid), clarithromycin (500 mg bid), and amoxicillin (1.0 mg bid) for one week. On the 8th week a second endoscopy was performed and further biopsy specimens were obtained from the same sites as in the initial endoscopy. One hundred and twenty-seven patients (69.1%) were found to be CagA positive and 57 patients (30.9%) were CagA negative. The total eradication rate was 82.6%. In the CagA-positive group this rate was 87.4%, and in the CagA-negative group it was 71.9% (P = 0.019). TNF-α levels were higher in the CagA-positive than in the CagA-negative group (P = 0.001). However, gastrin levels were not different between groups (P = 0.421). Our findings revealed that CagA-negative status might be a risk factor for failure of *H. pylori* triple therapies. The CagA pathogenicity island gives a growth advantage to *H. pylori* strains and has been associated with an increase in the inflammatory response at the gastric mucosal level. These properties could make CagA-positive *H. pylori* strains more susceptible to antibiotics.

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Introduction

Infection with *Helicobacter pylori* is a causal factor in the development of peptic ulcers (1). There is a strong association between gastritis induced by *H. pylori* and ulcer development, and peptic ulcer disease seldom develops in the absence of infection (1,2). Eradication of *H. pylori* leads to long-term remission and cure of peptic ulcer disease (2,3). The ability of *H. pylori* to cause ulcers appears to be linked to strain-specific characteristics, such as the presence of the cytotoxin-associated gene A (CagA) (1,4). It

has been reported that CagA-bearing *H. pylori* strains are more virulent and cause more severe gastroduodenal diseases (4,5) than CagA-negative strains.

Despite a multitude of clinical trials during recent years, no therapeutic regimen has clearly emerged as the optimal treatment for *H. pylori* infection. The success rates of the same eradication regimen reported by different centers show wide variability (6). One explanation for this variability may be different prevalence of infection with CagA-bearing strains according to geographic region. If CagA status affects the rates of response to treatment, then it may be possible to predict eradication rates previously and choose appropriate regimens for each patient according to whether the *H. pylori* strain is CagA positive or negative.

Thus, in the present study our objective was to evaluate the response to treatment for the eradication of *H. pylori* infection in CagA-positive and CagA-negative patients.

Material and Methods

The study was conducted according to good clinical practice and the Declaration of Helsinki. The study was approved by the local Ethics Committees before the enrollment of patients. Written informed consent was obtained from all patients.

Patients attended to routine diagnostic gastroscopy and likely to need *H. pylori* eradication therapy were recruited for this study. All patients belonged to the same mid-socioeconomic class with an annual income of US$ 2900 ± 120. All of them were from Manisa, a city in Western Turkey. The patients were also evaluated in terms of other epidemiological features like the number of rooms in the house, living place (city or country), educational level, gender, tooth brushing, smoking and alcohol use, but there was no significant difference between CagA-positive and -negative patients ($P>0.05$). One hundred and eighty-four patients (93 males, 91 females, mean age 42.6 ± 12.8 years) with chronic gastritis were studied. All of the 184 patients had *H. pylori* infection. Anti-*H. pylori* IgG (Pyloriset EIA-G, Orion Diagnostica, Espoo, Finland) was positive in all patients.

H. pylori positivity, a requirement to take part in the study, was determined by both the rapid urease test and histological examination. If one of these tests yielded negative results then this particular patient was not included in the study. Patients with previous gastric surgery, known bleeding diathesis, taking oral anticoagulants, or who had been treated with bismuth compounds, proton pump inhibitors, or antibiotics known to be active against *H. pylori* within the previous two months, were excluded from the study.

Subjects underwent an upper gastrointestinal endoscopy and biopsy specimens were taken from the gastric antrum, body, and fundus. Before the eradication therapy was given all patients were tested for CagA, serum tumor necrosis factor α (TNF-α) and gastrin levels. They were then prescribed lansoprazole (30 mg bid), clarithromycin (500 mg bid), and amoxicillin (1 g bid) for one week. During the 8th week a second endoscopy was performed and further biopsy specimens were obtained from the same sites as in the initial endoscopy. Compliance with medication was assessed by tablet counting and by direct questioning at the second endoscopy. Possible side effects of treatment were also assessed at these times by open and direct questioning, by the same gastroenterologist, using a standardized questionnaire.

To determine the presence of *H. pylori*, at pre-entry and by the 8th week, biopsy specimens were taken from the antrum (within 2 cm of the pylorus, two for histology and one for the rapid urease test), corpus (half way along greater curvature, two for histology), and fundus (high in the fundal vault, two for histology). An antral specimen was immediately placed in a tube for the rapid urease test. Biopsy specimens immersed

in formalin were sent to the pathology laboratory for histological examination. Formalin-fixed tissues were processed routinely, embedded in paraffin and cut into 5-µm sections. Along with the usual hematoxylin-eosin stain, all sections were also stained with toluidine blue in order to better reveal the bacteria. Slides were examined independently by two pathologists. Endoscopy was performed under sedation with 0 to 5 mg intravenous midazolam. An Olympus GIF1T-30 gastroscope was used and was thoroughly cleaned and disinfected between endoscopies. This involved internal and external brushing using a neutral detergent, washing for 7 min in an endoscope washer (model EW-20, Olympus Optical Company Limited, Tokyo, Japan) with neutral detergent, and 4-min disinfection with 2.2% glutaraldehyde.

Anti-CagA IgG antibodies (VIVA Diagnostika, Cologne, Germany, by ELISA) were used to determine the CagA status of *H. pylori* before treatment. Serum gastrin levels were studied by radioimmunoassay (double antibody gastrin, KGAD1, Diagnostic Products Corporation, Los Angeles, CA, USA). TNF-α levels were determined with an Amicyte kit by ELISA.

Patients with eradicated and non-eradicated *H. pylori* were compared according to their pretreatment CagA status. Fisher's exact probability test and the χ^2 test were used to determine the statistical significance. $P<0.05$ was considered to be significant.

Results

One hundred and twenty-seven patients (69.1%) were CagA positive and 57 (30.9%) were CagA negative. After the end of treatment patients were divided into two groups according to CagA status. The distribution according to age and gender was the same in both groups: 51 and 48.9% males, mean age 41.3 ± 11.2 and 43.3 ± 13.3 years in the CagA-positive and -negative groups, respectively ($P_1 = 0.280$, $P_2 = 0.390$).

After eradication therapy, 152 patients had no infection, but 32 still continued to be infected with *H. pylori*. The total eradication rate was 82.6%. *H. pylori* was eradicated in 111 of 127 (87.4%) CagA-positive patients and in 41 of 57 (71.9%) CagA-negative patients. CagA positivity seems to increase the eradication rates of *H. pylori* when one-week triple therapy is used. This difference was statistically significant ($P = 0.019$) (Figure 1).

H. pylori density was greater in the CagA-positive group (2.1 ± 1.0) than in the CagA-negative group (1.1 ± 0.6) ($P = 0.01$). *H. pylori* activity and chronic inflammation also were significantly higher in the CagA-positive group (1.6 ± 0.6 and 2.2 ± 0.9) than in the CagA-negative group (0.6 ± 0.3 and 1.2 ± 0.6, respectively) ($P = 0.001$, $P = 0.002$). The presence of atrophy and lymphoid aggregates was not different between the two groups ($P>0.05$). However, intestinal metaplasia was found to be significantly more frequent in patients infected with CagA-positive *H. pylori* strains ($P<0.001$).

TNF-α levels were also significantly different between CagA-positive and -negative groups (18.33 ± 4.7 *vs* 9.84 ± 2.3 pg/ml, respectively; $P = 0.001$) before treatment (Figure 2). Although gastrin levels seemed to be higher in the CagA-positive group than in the CagA-negative group, there was no statistical significance between these values, probably because of the wide range of standard deviation (56.7 ± 21.4 *vs* 47.5 ± 18.2 pg/ml; $P = 0.421$).

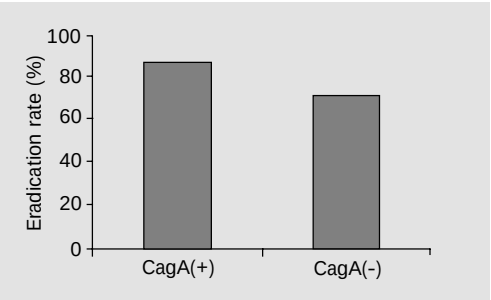

Figure 1. Eradication rates of the patients infected with cytotoxin-associated gene A (CagA)-positive and -negative *Helicobacter pylori* strains. $P = 0.019$ (Fisher's exact probability test).

Discussion

The gastroduodenal response to chronic *H. pylori* infection is characterized by the infiltration of plasma cells, lymphocytes, neutrophils and monocytes into the mucosa (7). Eradication studies have shown that this inflammatory response represents a specific reaction to the presence of *H. pylori* (6,7). In addition to stimulating local T and B cell responses and a systemic antibody response, *H. pylori* infection also induces a local pro-inflammatory cytokine response (8). There is now increasing evidence from both *in vivo* and *in vitro* studies that CagA-positive strains induce an enhanced inflammatory response and mucosal damage (6-8). The presence of CagA-positive *H. pylori* strains also significantly delays healing of ulcers (5). However, little information is available about whether this strain variability has any effect on the eradication rates of *H. pylori.*

In a study to evaluate the response to eradication treatment of *H. pylori* infection in CagA-positive and CagA-negative patients, the efficacy of the eradication treatment with triple therapy was found not to be dependent on the presence of the CagA gene (9).

In another study on the same subject from France (10), the authors tested whether the absence of CagA, which is more common in strains isolated from non-ulcer dyspeptic patients, was a risk factor for the failure of *H. pylori* eradication. Their results showed that the absence of *H. pylori* eradication was positively correlated with CagA-negative status (10). This result can explain the low efficacy of the current regimen when used in non-ulcer patients (10,11). Some recent data also indicate that eradication rates are higher for patients with CagA-positive *H. pylori* strains (12,13).

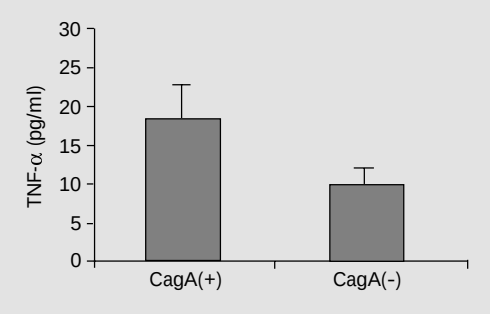

Figure 2. Serum TNF-α levels of cytotoxin-associated gene A (CagA)-positive and -negative groups. P = 0.001 (χ^2 test).

Strains of *H. pylori* expressing the CagA protein induce gastric epithelial cells to secrete inflammatory cytokines (14-16). Cytotoxins like bacterial cytotoxins, TNF-α, interleukin-1 and interleukin-6 were released from epithelial cells (16,17). In our study we used TNF-α and gastrin levels to determine the degree of chronic inflammation, as done in previous studies (18,19). TNF-α levels correlated with CagA positivity and showed enhanced inflammation by more virulent strains. There was no such correlation between gastrin levels and CagA status. The major limitation of our study was the measurement of antibody levels against CagA protein without the determination of the CagA protein itself. This is why we may have obtained some negative results due to some host factors that inhibit synthesis of an antibody against this specific protein. However, some data from the literature suggest that patients infected with CagA-negative *H. pylori* strains can produce anti-CagA antibody when infection with CagA-positive strains occurs (6,7).

We may speculate that treatments of longer duration may be necessary to increase the success rates of eradication treatment in patients infected with CagA-negative *H. pylori* strains (12,13). Another approach may be using some different combinations like three antibiotics plus a proton pump inhibitor or two antibiotics and a proton pump inhibitor plus bismuth citrate. Randomized, multicenter trials are needed to evaluate and determine the most effective and safe regimen and duration of treatment for these particular patients with CagA-negative strains. One may ask if CagA-negative strains, which are less virulent and more resistant to eradication therapy, should be necessarily eradicated, and if the indication of eradication

treatment should be different for CagA-negative and -positive strains.

Our findings revealed that CagA-negative status might be a risk factor for failure of *H. pylori* triple therapies. The CagA pathogenicity island gives a growth advantage to *H. pylori* strains and has been associated with an increase in the inflammatory response at the gastric mucosal level. These properties could make CagA-positive *H. pylori* strains more susceptible to antibiotics. Recommendations should be elaborated to manage these CagA-negative patients differently.

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