Gastroenterology and Hepatology From Bed to Bench.

ORIGINAL ARTICLE

The role of *Helicobacter pylori* and *CagA* in response to treatment in Iranian Gastroesophageal Reflux Diseases patients

Manouchehr Khoshbaten[1], Kaveh Baghaei[2], Yousef Bafandeh[3], Golam Reza Saeidi[1], Latif Gachkar[4], David Al Dulaimi[5], Reza Mahmoudi Lamouki[2], Mohammad Rostami Nejad [2], Mohammad Reza Bonyadi[1]

[1]*Liver and Gastrointestinal Diseases Research Center, Tabriz University of medical sciences, East Azerbaijan, Iran*

[2]*Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

[3]*Infectious Diseases and Tropical Medicine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

[4] *Infectious Diseases and Tropical Medicine Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

[5] *Department of Gastroenterology, Alexander Hospital Redditch, UK*

ABSTRACT

Aim: This study was conducted to evaluate the influence *H. pylori* infection and anti-*CagA* status on the efficacy of Omeperazole 20 m.g. b.d. for patients with endoscopic oesophagitis.

Background: The influence of *Helicobacter pylori (H. pylori)* infection and its virulent strain (cytotoxin-associated gene A: *CagA*) has not been evaluated on efficacy of treatment for patients with erosive oesophagitis in Iran.

Patients and methods: One hundred and ten patients (55 *H. Pylori* positive and 55 *H. Pylori* negative) with endoscopic evidence of oeosphagitis were enrolled in this interventional study and treated with Omeprazole 20 m.g. b.d. Healing was assessed at repeat endoscopy after 8 weeks of treatment. *H. Pylori* infection and anti-*CagA*-IgG (immunoglobulin G) antibodies were determined for each subject by the rapid urease test, pathological assessment and ELISA.

Results: At repeat endoscopy, following 8 weeks of Omeprazole 20 m.g. b.d. therapy, endoscopic healing of oesophagitis had occurred in 32 % of the HP +ve patients and 23 % of the HP –ve patients (chi square $p<0.01$). Among the HP +ve endoscopic healing occurred resolved in 11 (32.4 %) of the *CagA* +ve patients and 19 (90.5 %) of the *CagA* –ve patients. This difference was significant (chi-square $p <0.001$).

Conclusion: *H. pylori* infection and the *CagA* virulence factor are associated with an increased rate of healing amongst patients with endoscopic oesophagitis treated with Omperazole 20 m.g. b.d. compared to patients without *H. pylori* infection.

Keywords: Oesophagitis, *Helicobacter pylori* Cytotoxin-associated gene A, Omeperazole.

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Introduction

Helicobacter pylori (HP) is an important pathogen that is as an etiological factor in peptic ulcer disease (PUD), distal gastric cancer (DGC) and lymphoma (1, 2). The pathogenecity of HP infection is influenced by the presence of virulence factors. The major virulence factor for HP infection is thought to be cytotoxin-associated gene A (CagA). The presence of the *CagA*, a highly immunogenic protein, is associated with the ability of HP infection to cause peptic ulceration. HP infection can lead to an atrophic

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Reprint or Correspondence: Mohammad Rostami Nejad, PhD & Kaveh Baghaei, PhD. Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
E-mail: m.rostamii@gmail.com

fundal gastritis associated with a decrease in gastric acid production and a distal gastritis associated with increased gastric acid production (3).

Clinical manifestations of gastro-oesophageal reflux disease (GORD) include heartburn, regurgitation, and dysphagia. Patients with GORD are frequently treated with long-term acid suppression. GORD is a risk factor for oesophageal adenocarcinoma (OA). Factors that pre-dispose to GORD include incompetence of the lower oesophageal sphincter (LOS) and the presence of a hiatus hernia (HH). GORD can be diagnosed at endoscopy. The severity of oesophagitis at endoscopy can be classified according to the Los Angeles Classification (6). GORD can be treated with lifestyle modification, antacid therapy and anti-reflux surgery (7). Antacid therapy includes simple antacids; histamine class 2 receptor antagonists (H2RA) and proton pump inhibitors (PPIs) such as Omperazole.

Environmental factors, such as sanitation, may have a key role in the prevalence of HP in populations (8, 9). Epidemiological studies have shown that in Western populations the prevalence of HP infection is decreasing (10). This decrease in the prevalence of HP infection has been associated with a decrease in the prevalence of PUD and DGC. During the same time period there has been an increase in the in the prevalence of GORD and OA (11-13). Aetiological factors responsible for the increase in the prevalence of GORD may include obesity.

GORD is more prevalent in Western than in Asian populations, but studies have suggested that the prevalence of GORD in Asia is increasing (4, 14). The reasons for the increasing prevalence of GORD in Asia are uncertain, but it is speculated that this trend reflects the adoption of a western lifestyle in Asia, associated with a rise in the prevalence of obesity.

GORD is frequently associated with concurrent HP infection. The significance of HP infection in the pathogenesis of GORD remains uncertain. Some studies have reported that there is a correlation between increases in the prevalence of GORD and decreased prevalence of *H. pylori* infection. Other studies have failed to confirm this association (15-17).

Few studies have investigated HP and GORD in Iranian patients. This study was designed to the efficacy of Omeprazole (20 mg b.d.) therapy for the treatment of endoscopic oesophagitis in Iranian patients with evidence of HP infection (HP +ve) and without HP infection (HP –ve). Further analysis was performed for HP +ve patients according to the presence (HP +ve, *CagA* +ve) or absence (HP+ ve, *CagA* –ve) of serological evidence of the *CagA* virulence factor.

Patients and Methods

Patients were recruited following diagnostic gastroscopy for investigation of dyspeptic and reflux symptoms (symptoms present for at least six months) and endoscopic evidence of GORD as defined by the LA classification. Exclusion criteria included age less than 18 years, suspected pregnancy or lactation, endoscopic diagnosis of peptic ulcer disease or oesophageal candidiasis, previous peptic ulcer disease, previous oesophageal and gastric surgery, alcohol or drug dependency, intolerance of proton pump inhibitor and recent therapy with H2 antagonists or antibiotics (within one month of gastroscopy). An equal number of age-matched patients, with and without HP infection, were recruited. Informed consent was obtained from all patients and the protocol was approved by the hospital ethics.

Detection of *Helicobacter pylori*:

At endoscopy, two biopsy samples from the antrum and two samples from corpus of stomach were taken. *Helicobacter pylori* was detected

using both the Rapid Urease Test (RUT) and histopathological review of biopsies. For each patient one antral and fundal biopsy was tested for Hp infection using the RUT. If both samples were negative for HP infection antral and fundal biopsies were subject to conventional histopathological review and the presence of *H. pylori* noted. For histopathological review the biopsies were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and mounted on slides by means of standard technique. Slides were stained with hematoxylin-eosin and analyzed for the type and the condition of the epithelium. The biopsy specimens were evaluated for the presence of *H. pylori* infection using a Giemsa staining. Pathological review was performed by a senior histopathologist, unaware of subsequent treatment.

CagA detection by serotyping

2 ml blood sample in order to determination of IgG anti body titration against *CagA* subgroups and determination of the HP *CagA* status, was taken and after centrifuge in 4000 rpm serum samples are frozen at -70 for detection of IgG antibodies. Serum samples were assayed for *CagA* IgG antibodies serologic test was done in duplicate using by ELISA kit (CagA DIAGNOSTIC Kit, Italy). In keeping with manufacturer's instruction, recombinant *CagA* protein was used as a standard antigen. Dilutions were performed according to manufacturer's recommendation. 300-fold diluted serum was measured and a titer less than 15 units/mL was considered as a negative.

Treatment by Omeprazole

Following gastroscopy eligible patients were recruited and treated with omeprazole 20 mg b.d. After 8 weeks treatment, a repeat endoscopy was performed and the presence or absence of any oesophagitis was recorded. Oesophagitis was again classified according to on Los Angeles classification. Healing was defined as the absence of any endoscopic evidence of oesophagitis. The endoscopist was not aware of the HP or *CagA* status of patients. Outcomes were analyzed according to patient demographics, HP infection at diagnostic endoscopy and *CagA* serological status (among HP infected patients).

Statistical Analysis

Statistical analyses of the recorded data were performed by the chi-square -test and Fisher exact test and P values < 0.05 were accepted as significant relations.

Results

110 patients (70 males and 40 females, mean age of 49.5 years (range 19–80)) were successfully recruited. 55 patients were HP +ve and 55 HP –ve. All patients completed the study. The mean age of the HP +ve patients was 45.7 years and the mean age of HP –ve group was of 47.2 years. 34 of the 55 HP +ve patients (61.8%) had positive *CagA* serology. At baseline endoscopy the 55 HP +ve patients had oesophagitis with the following LA classification: Class I in 10 (18.2%), II in 32 (58.2%) and III in 13 (23.6%). At baseline endoscopy HP–ve patients had the following rates of endoscopic oesophagitis, according to LA classification: I in 10 (18.2%), II in 29(52.7%) and III in 16(29.1%). There was no significant difference in severity of oesophagitis between the HP +ve and Hp –ve patient groups (chi-square, $P > 0.05$) (table1). *H. pylori* has not important role against of GORD but it seems has a role in treatment HP+ ($x^2=13.41$, df=2 and $p<0.01$).

At baseline endoscopy the HP +ve *CagA* +ve and *CagA* –ve patients results revealed *cagA* has negative effective on cure procedure in gastroesophagiel reflux disease in patients with *Helicobacter pylori* infection($x^2=6.65$, df=1 and $p=0.009$). There was no significant difference when severity was compared according to *CagA* status.

At repeat endoscopy, following 8 weeks of omeprazole 20 mg b.d therapy, endoscopic healing

of oesophagitis had occurred in 32% of the HP +ve patients and 23 % of the HP –ve patients (table 2) (chi square p<0.01). Among the HP +ve endoscopic healing occurred resolved in 11 (32.4 %) of the *CagA* +ve patients and 19 (90.5 %) of the *CagA* –ve patients. This difference was significant (chi-square p <0.001).

Table1. The prevalence of oesophagitis in HP +ve and HP –ve according to LA classification before and after treatment

GERD	*N=110	0 (%)	I (%)	II (%)	III (%)
Before treatment	H.P +	------	10(18.2)	32(58.2)	13(23.6)
	H.P-	------	10(18.2)	29(52.7)	16(29.1)
After treatment	H.P+	30(54.5)	25(45.5)	------	------
	H.P-	23(41.8)	------	32(58.2)	------

* The number of patients in each group (HP+ and HP-) consists of 55 subjects

Table2. The prevalence of cagA antigen before and after treatment according to LA classification

*Grade		0 (%)	I (%)	II (%)	III (%)
Before treatment	CagA+	------	3(8.8)	21(61.8)	10(29.4)
	CagA-	------	7(33.3)	11(52.4)	3(14.3)
After treatment	CagA+	11(32.4)	23(67.6)	------	------
	CagA-	19(90.5)	2(9.5)	------	------

*$cagA^{+}$= 34; cagA- = 21

Discussion

Heartburn and acid regurgitation are two common symptoms of GORD. GORD symptoms frequently lead to specialist referral and endoscopic assessment. The reported prevalence of these symptoms in developing countries varies widely, but studies suggest a prevalence of over 50 % (18-20). The prevalence of HP infection in patients from developing countries with GORD varies widely and studies have reported values of between 30% and 90% (21, 10). Epidemiological studies suggest that the prevalence of GORD among Asian communities has increased, (22, 14, 4), but the prevalence remains lower than in Western populations. The pathophysiology of GORD is multifactorial. Identified factors include LOS competence and mucosal sensitivity (22). Diet and lifestyle may contribute to the development of GORD (23-25). Recent studies suggest the prevalence of GORD in Iran is increasing, in keeping with similar studies of Asian and Western populations (14, 26). It is hypothesized that this reflects the *'westernization'* of Iran and changes in the lifestyle and diet of its population. In this study two matched groups with endoscopic evidence of oesophagitis were treated with omperazole 20 m.g. b.d. following a diagnostic gastroscopy. There was no significant difference in the severity of oesophagitis at initial endoscopy. At repeat endoscopy, endoscopic healing of oesophagitis was associated with HP infection. Furthermore HP+ve *CagA* +ve infection was associated with a greater rate of oesophageal healing than HP +ve *CagA* –ve infection.

We suggest that among patients with endoscopic oesophagitis, HP infection is associated with an increased likelihood of endoscopic healing, following treatment with omperazole 20 m.g. b.d. Furthermore we suggest that this favourable outcome is associated with the presence of the *CagA* virulence factor. The mechanisms responsible for this improved rate of healing remains uncertain. We recommend that HP+ve *CagA* +ve infection may predispose to a reduction in gastric acid production. Alternatively infection may influence lower oesophageal sphincter function and promote oesophageal healing. Alternative explanations may include increased absorption of Omeprazole in the presence of *cagA* + HP infection.

After treatments status changed, it means we could get significant results from the experiments, between two groups (*H. pylori* positive & *H. pylori* negative) *H. pylori* positive group had a significant increasing to treatment response rather

than *H. pylori* negative group ($p<0.001$). This result was similar to other reports (27, 28). Our findings are in keeping with other studies that have suggested omeprazole treatment causes increased 24-h intragastric pH values in *H. pylori*-infected subjects than in *H. pylori* uninfected subjects (29, 30). Furthermore these studies suggest that *CagA* +ve HP infection is associated with increased intragastric pH compared to Hp +ve *Cag* –ve infection. If other studies confirm these findings they would support the hypothesis that HP infection may have a protective role, reducing the likelihood of developing GORD and increasing the efficacy of PPI therapy for GORD. Furthermore this hypothesis would suggest that HP infection should not be eradicated in the presence of endoscopic oesophagitis, in the absence of other indications such as PUD.

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