Treatment for Eradication of Helicobacter pylori Infection among Chronic Hepatitis C Patients

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Background/Aims: Helicobacter pylori infection causes gastritis, peptic ulcers and gastric malignancies, and its eradication has been advocated by many groups. We determined the H. pylori carrier status and eradication rates of patients with chronic hepatitis C virus (HCV) infection. Methods: In total, 76 chronically HCV-infected patients were enrolled for comparison with 228 HCV-noninfected, age- and sex-matched controls. H. pylori infection was confirmed by H. pylori antibody and urea breath testing. Results: The H. pylori infection rate was significantly higher for HCV-infected patients (67 of 76, 88.2%) than for HCV-noninfected controls (158 of 228, 69.3%). Endoscopic findings showed that the rates of gastric ulcers and gastritis were significantly higher for the 67 HCV-infected patients with H. pylori infection (34.3% and 77.6%) than for the 158 HCV-noninfected controls with H. pylori infection (15.2% and 57.6%). Treatment to eradicate H. pylori had a significantly higher success rate for HCV-infected patients (61 of 67, 91.0%) than for HCV-noninfected controls (115 of 158, 72.8%). Conclusions: The markedly high H. pylori eradication rate observed in this study shows that eradication of H. pylori holds promise for the improvement of the long-term health condition of patients with chronic HCV infection. (Gut Liver 2011;5:447-453)

Key Words: Helicobacter pylori; Chronic hepatitis C

INTRODUCTION

Helicobacter pylori (H. pylori) infection is one of the most common bacterial infections in the world. This infection is caused by spiral shaped gram-negative bacillus that colonizes the superficial area of the mucous gel layer of the human stomach. This bacterium has been implicated in the etiology of chronic active gastritis; peptic ulcer disease, gastric cancer and mucosa-associated lymphoid tissue lymphoma, and virtually all patients infected with H. pylori who have endoscopic biopsies are found to have histologic gastritis.

In 1994, the World Health Organization classified H. pylori as a grade I carcinogen, and approximately 50% of all humans infected with H. pylori. H. pylori infection increased significantly with age and male sex in our previous study. The prevalence of H. pylori infection increases with age but is quite different among various populations. H. pylori infection is more prevalent in groups of low socioeconomic status, so the overall antibody to H. pylori prevalence in developing countries is higher (60% to 80%) than in developed countries (40%). Most people infected with H. pylori probably acquired the infection in childhood when standards of living and sanitation, such as public water systems, were not well developed. A decrease in the frequency of a disease in successive generations (i.e., birth cohorts) is most likely due to changes in such environmental factors. In fact, the high prevalence (over 60%) of H. pylori in Japanese aged over 50 years is most likely due to poor environmental conditions during the period of turmoil just after World War II. Eradication of H. pylori is the recommended treatment to cure peptic ulcers and to prevent ulcer complications. Triple therapy with a proton pump inhibitor (PPI), clarithromycin (CAM), and amoxicillin (AMOX) is an effective regimen for the eradication of H. pylori.

Chronic hepatitis C virus (HCV) infection is a leading cause of end-stage liver disease worldwide. In Japan, the general prevalence of HCV infection is about 1%, but 50- to 70-year-old persons have a very high rate of infection. Chronic HCV infection causes several problems related to gastric and esophageal
diseases, such as hyper-portal gastritis and varices. It has been demonstrated that patients with liver cirrhosis are frequently subject to a number of disorders of the gastric mucosa, and peptic lesions in the gastroduodenal mucosa have been observed more often in cirrhotic patients than in controls. However, little information is available on the relationship of the gastroduodenal lesions and H. pylori infection of patients with chronic HCV infection. Also, there are very few studies of the H. pylori eradication rates for these patients.

To address the above issues, the present case control study was done to determine the persistent H. pylori infection rate and the eradication treatment response, by H. pylori-related endoscopic findings, of patients with chronic HCV infection and a control group of HCV-uninfected persons.

MATERIALS AND METHODS

1. Patients

The present study consisted of 76 patients with chronic HCV infection and 228 HCV-noninfected control subjects living in Fukuoka who received esophagogastroduodenoscopy in Kyushu University Hospital between January 2005 and January 2010. Of the 76 HCV infected patients, none had hepatic cell carcinoma or severe liver dysfunction such as ascites or hepatic encephalopathy. Also, 23 patients had histologically confirmed cirrhosis of Child-Pugh class A, but none were of Child-Pugh classes B or C. The present study was a part of an epidemiological study of the changes of H. pylori infection prevalence in the Japanese general population that surveyed over 4,700 residents who participated in a general health checkup program over the last 2 decades. Over 1,000 participants with antibody to H. pylori (anti-H. pylori) positive in the ongoing study who visited our hospital to check for persistent H. pylori infection underwent endoscopic examination. Of them, 228 age and sex matched HCV-noninfected control subjects were randomly selected for comparison.

To ensure the validity of the data, all doctors carrying out the study were staff members of our department trained in the study protocol. All patients and controls were given a thorough medical history interview, clinical examination, abdominal ultrasound and laboratory investigations, including complete blood counts, total bilirubin (TB), serum total protein (TP), serum albumin (Alb), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum creatinine, and prothrombin time (PT) at a commercial laboratory (MBC Laboratory, Tokyo, Japan). Hepatitis B surface antigen (HBsAg) and antibody to HCV (anti-HCV) were tested by commercial enzyme-linked immunosorbent assay (ELISA) kits and reverse transcriptase polymerase chain reaction assay for serum HCV RNA (COBAS Amplicor HCV Monitor Test v2.0; Roche Diagnostics, Tokyo, Japan). All patients were positive for anti-HCV and HCV RNA and negative for HBsAg. All control subjects were negative for both HCV and HBV infection. Persistent H. pylori infection was defined as positive for serum anti-H. pylori and 13C-urea breath test (UBT). All patients and control subjects were interviewed by our staff doctors about the presence of dyspepsia (bothersome postprandial fullness, early satiation, epigastric pain, and epigastric burning), based on the Rome III criteria. The dyspeptic symptoms had to be present for more than 3 days a week for at least 6 months. Informed consent was obtained from all patients and controls before enrollment. The present study was carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000.

2. Persistent H. pylori infection

Serum samples were collected from all patients and control subjects, separated and stored at -80°C until testing for routine serum biochemistry. Anti-H. pylori was measured in serum using an ELISA (E plate Eiken Disk H. pylori antibody; Eiken Chemical Co., Tokyo, Japan). An antibody concentration ≥10.0 ELISA value in the assay indicates anti-H. pylori IgG positivity.

Urea breath test to confirm persistent infection was done only for patients and control subjects with anti-H. pylori positive. Patients and control subjects fasted for at least six hours before this test, after which a first breath sample was collected. A 100 mg 13C-urea tablet (UBITkit; Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) was then administered orally, and a second breath sample was collected 20 minutes later. The collected samples were analyzed using an isotope-selective nondispersive infrared spectrometer (UBIT-IR300; Otsuka Pharmaceutical Co., Ltd.). The cut-off value of ECR used for H. pylori eradication was 2.5‰. The urea breath test was used for the diagnosis of persistent H. pylori infection at enrollment and to confirm successful eradication.

3. Esophagogastroduodenoscopy

All patients and control subjects underwent esophagogastroduodenoscopy at enrollment. Four experienced endoscopists of our hospital performed each examination without knowledge of any data of the studied patients. Endoscopic diagnosis included erosive reflux disease, gastritis, gastric ulcer, duodenal ulcer, and gastric cancer, based on the judgment of the four endoscopists.

4. Histological examination

Needle biopsy of the liver was done only for 76 patients with chronic HCV infection. Two pathologists did the histopathological assessment separately, and then a consensus between them was made on discordant assessments. The pathologists were not aware of the clinical data, at the time of assessment. The MetaMorpheus fibrosis score was used to evaluate histological fibrosis from F0 to 4: F0, no scarring; F1, minimal scarring; F2, scarring has occurred and extends outside the areas in the liver that contains blood vessels; F3, bridging fibrosis is spreading and connecting
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...to other areas that contains fibrosis; F4, cirrhosis or advanced scarring of the liver. Of the 76 patients, none, 20, 19, 14, and 23 were assigned as F0, F1, F2, F3, and F4, respectively.

No histological examination was done for HCV-noninfected controls. For the HCV-noninfected controls, the presence or absence of cirrhosis was judged by the results of the following markers: platelet counts, PT, and serum levels of TP, Alb, TB, AST, ALT, ALP, and type IV-collagen.

5. H. pylori eradication protocol

The triple drug combination therapy for H. pylori eradication included lansoprazole 60 mg b.i.d., clarithromycin (CAM) 800 mg b.i.d., and amoxicillin (AMOX) 1,500 mg b.i.d. for seven days. The successful eradication of H. pylori was defined as negative for urea breath test at 24 weeks after the therapy (Fig. 1). Patients and control subjects were instructed to refrain from any antibiotics for at least four weeks and from PPIs for at least eight weeks before testing for the eradication response to minimize the chance of false negative results by urea breath test.

6. Statistical analysis

The rate of successful H. pylori eradication was determined by intention-to-treat and per-protocol analysis. Statistical analysis was done with SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL, USA). Continuous data were expressed as mean±standard deviation. Continuous variables were analyzed using the Student’s t-test. Categorical variables were analyzed using the chi-square test, Fisher’s exact test, or trend test. A p-value of less than 0.05 was regarded as statistically significant.

RESULTS

Table 1 shows the characteristics of the 304 studied patients and control subjects. The persistent H. pylori infection rate was significantly higher for the 76 patients with chronic HCV infection (88.2%) than for the 228 HCV-noninfected controls (69.3%) (p=0.0020). No significant differences in the H. pylori infection rate were found among the 76 HCV-infected patients with F0-1 (n=20), F2-3 (n=33), and F4 (n=23) classifications by histological fibrosis; 90.0%, 87.8%, and 86.9%, respectively. The frequency of dyspeptic symptoms did not significantly differ between the patients with chronic HCV infection (9 of 76, 11.8%) and the HCV-noninfected controls (25 of 228, 10.9%).

Table 2 shows the clinical characteristics of 76 patients with chronic HCV infection, classified by H. pylori infection status. No significant differences were found in sex, age, HCV viral markers, or other parameters between patients with and without H. pylori infection.

Table 1. Clinical Characteristics of 76 Patients with Chronic HCV Infection and 228 Participants without HCV Infection

| Characteristics                        | Chronic HCV infection | p-value |
|----------------------------------------|-----------------------|---------|
|                                       | Yes (n=76)            | No (n=228) |       |
| Male, n (%)                            | 42 (55.3)             | 126 (55.3) | Matched |
| Age, yr                                | 58.1±9.9              | 58.1±9.9 | Matched |
| HCV genotype 1b, n (%)                 | 49 (64.5)             | -        | -       |
| HCV RNA level, kIU/mL                  | 1,892.3±235.9         | -        | -       |
| HCV RNA level under 100 kIU/mL, n (%)  | 4 (5.3)               | -        | -       |
| Alanine aminotransferase, IU/L         | 90.2±12.5             | 23.6±7.9 | <0.0001 |
| Platelet count, ×10^4/μL               | 12.4±2.0              | 25.8±6.1 | <0.0001 |
| Liver cirrhosis, n (%)                 | 23 (30.3)             | 0        | <0.0001 |
| Dyspepsia, n (%)                       | 9 (11.8)              | 25 (10.9) | 0.835   |
| Persistent Helicobacter pylori infection, n (%) | 67 (88.2) | 158 (69.3) | 0.002   |

Chronic HCV infection was defined as serum positivity for antibody to HCV and HCV RNA. Persistent Helicobacter pylori infection was defined as positivity for the antibody and urea breath test. Continuous data are shown as the mean±SD. HCV, hepatitis C virus.

Fig. 1. Protocol for Helicobacter pylori eradication treatment.
Table 3 shows the endoscopic findings of the patients and controls, classified by *H. pylori* and HCV infection status. The rates of gastritis and gastric ulcer were significantly higher for the 67 HCV-infected patients with *H. pylori* infection (group A) (77.6% and 34.3%) than for the 158 HCV-noninfected controls with *H. pylori* infection (group B) (57.6% and 15.2%) (all p<0.05) and for the 70 HCV-negative controls without *H. pylori* infection (group D) (32.8% and 4.3%) (all p<0.05). Classified by histological fibrosis, no significant differences were found in the rates of endoscopic findings of HCV-infected patients: 75.0%, 72.7%, and 73.9% for gastritis; 35.0%, 30.3%, and 26.1% for gastric ulcer; and 10.0%, 12.2%, and 8.7% for duodenal ulcer for the F0-1 (n=20), F2-3 (n=33), and F4 (n=23) stages, respectively.

All of the 67 HCV-infected patients and 158 HCV-negative controls with *H. pylori* infection agreed to receive the eradication treatment after informed consent. The treatment was discontinued by three of the HCV-infected patients (2 diarrhea, 1 erupption) and nine of the controls (3 diarrhea, 2 taste disorder, 1 erupption, and 3 who dropped out). In an intention-to-treat analysis, the successful eradication rate of 91.0% (61 of 67) was significantly higher for the HCV-infected patients than the rate of 72.8% (115 of 158) for the controls (p=0.0043). Classified by stage of histological fibrosis, the eradication rates increased with the fibrosis score of HCV-infected patients: F0-1 (83.3%, 15 of 18), F2-3 (93.1%, 27 of 29), and F4 (95.0%, 19 of 20), with no significant differences. The per-protocol analysis also showed a significant difference in the rate of successful eradication between the HCV-infected patients (61 of 64, 95.3%) and the controls (115 of 149, 77.2%) (p=0.0026).

**DISCUSSION**

The present study showed a markedly higher rate of *H. pylori* infection and higher rates of gastric ulcer and gastritis for HCV-infected patients than for patients without HCV infection. Moreover, the rate of successful eradication was found to be markedly higher for the HCV infected patients than for the control patients without HCV infection. One Japanese report showed that coexistent *H. pylori* infection did not influence the clinical course of chronic hepatitis C. To our best knowledge, this is the first study to demonstrate such a markedly high *H. pylori* infection rates.

### Table 3. Differences in Endoscopic Findings and Eradication Treatment Rates of 76 HCV-Infected Patients and 228 HCV-Uninfected Controls, Classified by *Helicobacter pylori* and HCV Infection Status

| Group | Persistent *Helicobacter pylori* infection | Chronic HCV infection | No. | Chronic gastritis prevalence, n (%) | Gastric ulcer prevalence, n (%) | Duodenal ulcer prevalence, n (%) | Eradication treatment rate*, n (%) |
|-------|------------------------------------------|-----------------------|-----|------------------------------------|--------------------------------|----------------------------------|----------------------------------|
| A     | Yes                                      | Yes                   | 67  | 52 (77.6)                          | 23 (34.3)                      | 8 (11.9)                         | 61 (91.0)                        |
| B     | Yes                                      | No                    | 158 | 91 (57.6)                          | 24 (15.2)**                    | 12 (7.6)                         | 115 (72.8)                       |
| C     | No                                       | Yes                   | 9   | 4 (44.4)†                          | 0†                             | 0†                              | 0†                              |
| D     | No                                       | No                    | 70  | 23 (32.9)                          | 3 (4.3)**                      | 0                               | 0                               |

*Groups A and C consisted of 76 patients with chronic HCV infection. Persistent *Helicobacter pylori* infection was defined as positivity for the antibody and urea breath test. Chronic HCV infection was defined as positivity of antibody to HCV and HCV RNA in the serum.

**HCV, hepatitis C virus.**

*†Significantly higher than Group B patients (p=0.0043); † vs †, p=0.0069; † vs †, p=0.484; † vs †, p=0.0001; † vs †, p=0.0023; † vs †, p=0.0503; † vs †, p<0.0001.
eradication rate for patients with HCV infection.

In comparison with worldwide data, the anti-*H. pylori* prevalence for the age groups under 40 years of our previous Japanese report was low (about 20%) and similar to those in the USA, France, the Netherlands, Finland, Wales, Australia, and other developed countries, but the prevalence in the age groups over 50 years were high (over 60%) and similar with those in Algeria, Ivory Coast, Vietnam, Thailand, Papua New Guinea, and Peru, which are developing countries. In brief, most of our studied persons aged 50 or over years had a high probability of having *H. pylori* infection in this area where the infection was historically widespread.

The most likely mode of *H. pylori* transmission is from person to person, by either the oral-oral (through vomit or possibly saliva) or fecal-oral route. Also, the iatrogenic transmission of *H. pylori* or following endoscopy (gastric oral route) is common if the proper method of disinfection is not followed. *H. pylori* infection also has a worldwide distribution. Most transmission of *H. pylori* is through the transfusion of blood or blood products, the transplantation of organs from infected donors, and through the sharing of contaminated needles among injection-drug users. Sexual transmission has also been well documented. Although *H. pylori* and HCV have entirely different routes of transmission, we found a higher rate of *H. pylori* infection among patients with chronic HCV infection than among HCV-uninfected persons. Usually, most *H. pylori* infection is acquired during childhood, while HCV infection occurs at any age. The following hypothesis might explain the higher *H. pylori* infection rate of patients with HCV infection in the present study. Patients with chronic HCV infection display impaired virus-specific CD4+ and CD8+ T cells with lower proliferative and gamma interferon (IFN γ)-producing capacities, which leads to T cell dysfunction. This may contribute to increased susceptibility to secondary microbial pathogens, including viruses, bacteria, and parasites, thus possibly increasing the risk of *H. pylori* infection. *H. pylori* possibly evades host responses through the inhibition of antigen specific T cell proliferation, major histocompatibility complex class II-restricted, cell-mediated mechanisms that control *H. pylori* infection, or adoptive transfer of CD4+ T cells that have been demonstrated to have a protective role of T cells. In spite of good host immune responses, some bacteria are not cleared and a chronic infection can be established.

We found significantly higher rates of gastric ulcer and gastritis for HCV-infected patients with *H. pylori* infection than for HCV-negative persons with *H. pylori* infection. In the late 1980s, it became apparent that there was a very close association between chronic active gastritis and colonization with *H. pylori*. The major genetic determinant of *H. pylori* virulence is the cag Pathogenicity Island (cag PAI), which encodes a type IV secretion system that allows an immunodominant antigen, CagA, to be translocated into gastric epithelial cells. In comparison to infection with cag PAI-negative *H. pylori* strains, infection with cag PAI positive strains is associated with an increased severity of gastric mucosal inflammation, an increased risk for the development of peptic ulceration, and an increased risk of gastric cancer. It is important that *H. pylori* infection can alter acid secretion in both directions. Meanwhile, chronic liver disease has several problems related to gastric and esophageal diseases, such as hyperportral gastritis and esophageal and gastric varices. The most important pathogenesis factor of gastroduodenal lesions in end stage liver disease and cirrhosis is portal hypertension. This causes splenic congestion, which, at least in theory, might interfere with the normal reparative processes of the gastroduodenal mucosa, leading to increased susceptibility to acid and pepsin secretion. Changes in gastric microcirculation in cirrhosis, such as increased straight arterioles and dilated pre-capillaries as well as capillaries and veins, have been reported. These alterations are probably related to portal hypertension and might contribute to acid peptic lesions. However, there was no significant difference in the rates of gastric ulcer and gastritis between our cirrhotic and noncirrhotic HCV infected patients with *H. pylori* infection, possibly because our study was too small to clarify the difference.

Eradication of *H. pylori* is very efficacious in the treatment of upper gastrointestinal disease. The standard strategy for the cure of *H. pylori* infection includes a triple drug combination therapy of a PPI, AMOX, and CAM or metronidazole but the development of resistance by *H. pylori* to the latter two drugs has been reported. Eradication success depends mainly on the patient’s adherence to treatment and bacterial resistance to medications. In Japan, CAM consumption multiplied by four between 1993 and 2000. This can be explained by the widespread use of CAM in Japan, suggesting resistance to the drug. The present study showed successful eradication rates of 91.0% for HCV-infected patients and 72.8% for HCV-uninfected controls by intention-to-treat analysis. This result might be interpreted by the role of liver disease on the metabolism of PPI. A PPI is an important drug in the triple therapy used for the eradication of *H. pylori* because of its action in inhibiting acid formation by blocking [H(+)/K(+)]-ATPase of the stomach parietal cells after being transformed into an active form under acidic conditions. Its catabolism in the liver depends mainly on an isoenzyme of cytochrome P450 2C19. It has two genetically determined phenotypes in humans: extensive metabolizers and poor metabolizers. Phenotype variation affects the acid suppressing effects of omeprazole by changing its rate of catabolism. The frequency of the poor metabolizer genotype is much higher in Japanese populations (18.8%) than in Caucasian populations (2.1%). Some studies have shown that PPI metabolism is affected by many factors, such as gender, age and concomitant use of other drugs. These factors are usually associated with a decrease in liver function. Although it is very hard to determine the precise mechanism, the disorder of liver function by chronic HCV infection might be from a decrease in the PPI metabolism,
which may explain the high eradication rate of *H. pylori* of HCV-infected patients.

The present study has certain limitations. First, screening of *H. pylori* infection was determined only by serum antibody. The first aim of this study was to investigate persistent *H. pylori* infection in HCV-infected patients defined by a urea breath test with a higher sensitivity and specificity. Therefore, further studies are needed to find an association between anti- *H. pylori* negativity and urea breath test positivity among HCV-infected patients. Second, no information on antibiotic resistance to *H. pylori* was available. We did not perform *H. pylori* culture or urea breath test positivity among HCV-infected patients. Their assistance with the present study.

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**CONFLICTS OF INTEREST**

No potential conflict of interest relevant to this paper was reported.

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