ORIGINAL PAPER

DOI: http://dx.doi.org/10.15403/jgld-4238

Helicobacter Pylori Colonization Density is an Important Risk Factor for Eradication Therapy

Hakan Demirci[1], Kadir Ozturk[2], Alpaslan Tanoglu[3], Ayse Guldem Kilciler[4], Armagan Gunal[5], Mustafa Gulsen[4], Ahmet Uygun[4]

1) Department of Gastroenterology, Medical Park Hospital, Istanbul;
2) Department of Gastroenterology, Liv Hospital, Ankara;
3) Department of Gastroenterology, University of Health Sciences, Sultan Abdulhamid Han Hospital, Istanbul;
4) Department of Gastroenterology, Healty Sciences University, Gulhane Training and Research Hospital, Ankara;
5) Department of Pathology, University of Health Sciences, Gulhane Training and Research Hospital, Ankara, Turkey

ABSTRACT

Background & Aims: Despite the known risk factors, it is not clear why the same treatment protocol for *Helicobacter pylori* infection (*H. pylori*) does not show a similar effect in patients with common risk factors. We hypothesized that as the severity of *H. pylori* - induced gastric mucosa inflammation and density increase, the rate of successful treatment decreases. This study aimed to explore the existence of a possible association between gastric *H. pylori* colonization density and the efficacy of bismuth-containing quadruple eradication therapy.
Methods: A total of 330 patients with *H. pylori* positive gastritis were initially included; the diagnosis was based on the histopathological examination. *H. pylori* colonization density was graded according to the Sydney classification: mild (n=101), moderate (104) and severe (98). *H. pylori* eradication was determined via the ^{13}C-Urea breath test performed eight weeks after therapy.
Results: There was no significant difference in terms of the distributions of age, gender, alcohol consumption, and smoking status among the groups (p>0.05). The successful eradication rates of *H. pylori* were 87.1%, 78.8%, and 75.5%, respectively, for the mild, moderate, and severe *H. pylori* colonization groups by per-protocol analysis (p=0.038). The eradication rates of *H. pylori* were 81.5%, 73.2%, and 67.3% respectively, for the mild, moderate, and severe *H. pylori* colonization groups by intention-to-treat analysis (p=0.017).
Conclusions: *Helicobacter pylori* colonization severity might predict the usefulness of eradication therapy in pre-treatment assessment. We recommend the use of more effective therapy regimens for *H. pylori* eradication in patients with severe densities.

Key words: *Helicobacter pylori* – *H. pylori* – colonization density – quadruple therapy – eradication.

Abbreviations: *H. pylori*: *Helicobacter pylori*; ITT: intension to treat; PP: per protocol; PPI: proton pump inhibitor; RUT: rapid urease test; UBT: ^{13}C-Urea breath test.

Address for correspondence:
Kadir Ozturk, MD
Associate Professor,
Liv Hospital, Ankara, Turkey
kadirozturk3041@gmail.com

Received: 26.01.2022
Accepted: 18.04.2022

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is currently the most commonly diagnosed chronic bacterial gastric infection worldwide. While it has been diagnosed in 20–50% of the general population in Western countries, its prevalence has been reported to reach nearly 80% in developing countries [1–3]. This bacteria, which occurs frequently in all populations, has a wide spectrum of manifestation from asymptomatic infection to serious diseases, such as malignancy [4–5]. Many guidelines recommend the test-and-treat strategy for uninvestigated dyspepsia in patients with no alarm symptoms in areas where the *H. pylori* prevalence is high (> 20%) [6–7]. Many challenges are encountered for various reasons throughout the treatment period.

Among the challenges, treatment failure of *H. pylori* is a major concern that needs to be considered in the management of *H. pylori* infection. As in many infectious diseases, the main cause of treatment failure is antibiotic resistance [8]. It is well known that the overprescribing and widespread use of certain antibiotics in daily practice contributes to increased *H. pylori* resistance [9–10]. In addition, smoking, obesity, and rapid proton pump inhibitor (PPI) metabolism have also been reported as risk factors for *H. pylori* eradication failure [11–12]. Despite these known risk factors, it is not clear why the same treatment protocol does not show a similar effect in patients with common risk factors. Previous studies reported that a higher gastric *H. pylori* colonization density negatively affected

the successful rates of triple eradication therapy [13–15]. The Sydney classification is the most used method to detect the severity of *H. pylori* colonization in clinical practice. The endoscopic Kyoto classification is another useful method for assessing *H. pylori* gastritis and is consistent with the Sydney classification [16]. We hypothesized that as the severity of *H. pylori*-induced gastric mucosa inflammation and density increase, the rate of successful treatment decreases.

Due to the above-mentioned challenges in *H. pylori* treatment, many therapy protocols from different regions are recommended. In the Maastricht IV/Florence Consensus Report [17] on the management of *H. pylori* infection, quadruple therapy containing metronidazole and bismuth is recommended as the first-line eradication treatment in populations with high clarithromycin resistance (>15–20%). Therefore, this protocol was used for our study population. We aimed to explore the existence of a possible association between gastric *H. pylori* colonization density and the efficacy of bismuth-containing quadruple eradication therapy (colloidal bismuth subcitrate 300 mg q.i.d., rabeprazole 20 mg b.i.d., tetracycline 500 mg q.i.d., and amoxicillin 1000 mg b.i.d. for 14 days).

METHODS

This single-center study was carried out at the Gastroenterology Clinic of Gulhane Medical Academy, Turkey. Ethical approval was obtained from the local Ethical Committee (50687469-1491-133/ February 24, 2014) and each subject provided written informed consent to participate in the study. Patients were excluded from the study if they met any of the following criteria: a history of gastric cancer, surgery, allergy to antibiotics, renal or liver failure, pregnancy, and immune-compromised patients. Patients who had been treated with bismuth, PPIs, H2 receptor blockers, antioxidant vitamins, such as vitamin C and E, probiotics, prebiotics or antibiotics within the previous month were also excluded to avoid interference with the eradication of *H. pylori*. Patients with dyspeptic symptoms and without ulcers on endoscopic examination were included in the study. To diagnose the *H. pylori* infection, a rapid urease test (RUT) (GrupoBios product, Chile) and a histopathological evaluation were performed. Subjects were deemed infected with *H. pylori*when both tests were positive.

A total of 330 *H. pylori* infected patients met our inclusion criteria. All participants were divided into three groups according to Sydney classification (mild, moderate, and severe). All subjects received bismuth-based quadruple therapy, recommended as the first-line treatment protocol in regions where *H. pylori* resistance to clarithromycin exceeds 15–20%. Our treatment protocol was as follows: amoxicillin (1000 mg b.i.d.), tetracycline (500 mg q.i.d.), bismuth subcitrate (400 mg b.i.d.), and rabeprazole (20 mg b.i.d.) for 14 days. At the end of the study, 101 patients (53 male) with mild *H. pylori* colonization, 104 (50 male) with moderate *H. pylori* colonization, and 98 (50 male) with severe *H. pylori* colonization completed all the procedures. The eradication rate of *H. pylori* was determined by a ^{13}C-Urea breath test (UBT) performed at least four weeks after the treatment (cut-off value: +3 delta/1000 for positivity). ^{13}C-UBT was performed after an overnight fast. Before testing, PPIs and antibiotics were discontinued for at least two and four weeks, respectively. A negative ^{13}C-UBT result was described as successful eradication.

All endoscopic procedures were performed by the same gastroenterologist. An Olympus GIF-Q165 endoscope was used. The biopsy forceps were WG Endo-Technique oval type, tapered and fenestrated (2.2 mm diameter and 180 cm length). During the upper gastrointestinal endoscopy, one biopsy sample from the antrum was first obtained for a RUT. We decided to take a biopsy for histopathologic analysis if the RUT was positive within five to ten minutes. Two biopsies from the antrum (lesser and greater curvature of the antrum within 2–3 cm of the pylorus) and two biopsies from the corpus (lesser and greater curvature of the body 8 cm from the cardia) were taken.

All obtained biopsies were fixed in 10% neutral formalin and processed according to standard methods. Sections were prepared at a thickness of three micrometers. The biopsy samples were stained with hematoxylin-eosin and a modified Giemsa stain. Two experienced pathologists examined all the samples. The updated Sydney system was used to grade the samples. Grading gastric mucosal inflammation was based on the intensity of mononuclear inflammatory cellular infiltrates within the lamina propria: absent (0), mild (1), moderate (2), or severe (3). The density of *H. pylori* colonization was graded as mild, moderate, or severe according to the Sydney classification [18]. If there was a difference between the two pathologists and/or specimens in terms of density, the highest grades were selected.

All statistical analyses were performed using SPSS software (Statistical Package for the Social Sciences, version 16.0, SSPS Inc., Chicago, IL, USA). Continuous variables were given as mean ± standard deviation and compared via the Student's *t*-test, depending on the normality of distribution. Categorical variables were calculated as percentages and frequencies and compared statistically using a Chi-square test. The eradication rates of *H. pylori* according to the density were compared using the Chi-square test or Fisher's exact test. Per-protocol (PP) analysis and intention-to-treat (ITT) analysis were performed. The 95% confidence intervals were calculated. A p value of < 0.05 was considered significant.

RESULTS

A total of 330 patients were initially enrolled. The average ages of the patients were 47.9±10.5, 48.1±10.1, and 47.5±11.2 years, respectively, in the mild, moderate, and severe groups. The distributions of age, gender, alcohol consumption, and smoking status was similar among the groups (Table I). 92% (n=303) completed the study; seven patients (2.1%) interrupted the treatment protocol due to intolerable adverse effects: epigastric pain (1%), diarrhea (0.3%), nausea (0.4%), and vomiting (0.4%) and twenty patients (6%) were lost to follow-up (Fig. 1).

The successful eradication rates of *H. pylori* were 87.1%, 78.8%, and 75.5%, respectively, for the mild, moderate and severe *H. pylori* colonization groups by PP analysis (p=0.038).

Table I. Comparison of *H. pylori* eradication rates between groups according to the Sydney classification.

	Mild	Moderate	Severe	p
Age, years (mean±SD)	47.9±10.5	48.1±10.1	47.5±11.2	0.940
Male (%)	52.5	48.1	51	0.814
Smoking (%)	19.8	21.2	20.4	0.972
Alcohol (%)	8.9	11.5	10.2	0.613
Eradication Rate (%)				
Per Protocol (PP)	87.1	78.8	75.5	0.038
Intention to Treat (ITT)	81.5	73.2	67.3	0.017

Binary comparisons for PP: p=0.115 for group mild vs moderate, p=0.572 for group severe vs moderate, p=0.035 for group mild vs severe. For ITT: p=0.144 for group mild vs moderate, p=0.333 for group severe vs moderate, p=0.016 for group mild vs severe; SD: standard deviation.

The eradication rates of *H. pylori* were 81.5%, 73.2%, and 67.3% respectively, for the mild, moderate, and severe *H. pylori* colonization groups by ITT analysis (p=0.017). In binary comparisons of groups, a significant difference existed between the severe and mild groups (p=0.0035 PP analysis; p=0.016 ITT analysis). Multivariate analysis revealed that a high *H. pylori* density was an independent risk factor for eradication failure (p=0.012 PP analysis; p=0.032 ITT analysis). However, no significant difference was found in the other comparisons (Fig. 2).

DISCUSSION

In this study, bismuth-based quadruple therapy had a high eradication rate for *H. pylori* as a first-line therapy in our population. The density of *H. pylori* colonization was associated with eradication success, this finding being the main result of our study. The eradication rate of *H. pylori* decreased as the density of *H. pylori* colonization increased. However, even if numerical differences were observed among groups, a statistically significant difference existed only between the severe and mild groups in both the ITT and PP analyses.

The Maastricht IV/Florence Consensus Report recommends bismuth-containing quadruple therapy as the first-line *H. pylori* eradication therapy in areas of high clarithromycin resistance [13]. Our *H. pylori* study group previously reported high efficacy of bismuth-containing quadruple therapy as a first-line treatment option for *H. pylori* in our population [19–21]. It is important to use a highly effective therapy option to better evaluate the effects of other factors except the treatment protocol. Therefore, in this study, we preferred the use of bismuth-containing quadruple therapy for first-line *H. pylori* eradication therapy. Previous studies reported different eradication rates for *H. pylori* despite using the same treatment protocols. Many authors investigated other factors except the antibiotic resistance that might influence the *H. pylori* eradication success rate, such as genetic factors, concomitant medication use, smoking, and diabetes [22]. None of the guidelines recommends considering the *H. pylori* colonization density when choosing an eradication regimen. However, some authors from our region hypothesized that *H. pylori* density or inflammation severity could be risk factors for treatment failure. In 2013, Onal et al. [23] reported that the success of triple eradication therapy containing clarithromycin and amoxicillin was negatively affected by the gastric colonization density of *H. pylori*. However, they could not demonstrate the same association with the group treated with quadruple treatment containing colloidal bismuth subcitrate, lansoprazole, tetracycline, and metronidazole. In their study, the eradication rate of *H. pylori* was 50% in the triple therapy group and 92% in the quadruple therapy group. This significant difference of the eradication rates might explain the inconsistent results. In addition, their study population for quadruple eradication therapy was limited to 26 patients which might explain the variance in the results of these two

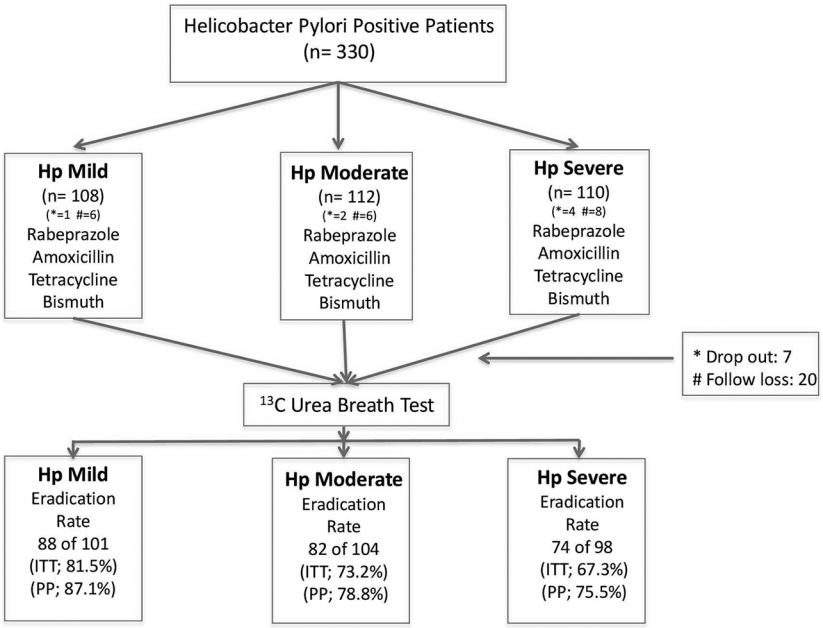

Fig. 1. Patients' distribution and eradication rates.

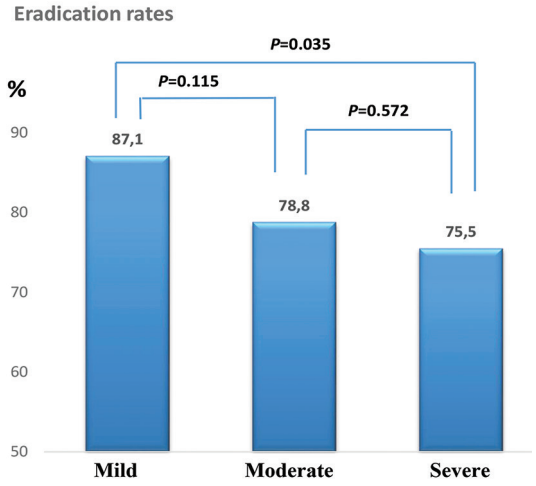

Fig. 2. Binary comparisons of groups for per-protocol analysis.

protocols. In another study, Kalkan et al. [24] concluded that *H. pylori* density was not a risk factor for eradication failure in subjects who received quadruple treatment containing colloidal bismuth subcitrate, rabeprazole, tetracycline, and metronidazole. Despite these disappointing results, we still hypothesized that there is a relationship between the bacterial density and the eradication rate due to our clinical practice observations. Therefore, we created a special homogeneous group with enough patients, and used Sydney classification rather than the above-mentioned studies. We found distinct eradication rates among mild, moderate, and severe groups in the PP analysis: 87.1%, 78.8%, and 75.5%, respectively. A significant difference existed between the severe and mild groups. Although the difference between the moderate and mild groups was not significant, we know that a decreased eradication rate below 80% in most countries is unacceptable for *H. pylori* treatment [25]. In this context, statistical insignificant results should not be interpreted as being clinically unimportant

To date, several clinical studies from different regions have reported that increased *H. pylori* colonization density was closely related to peptic ulcer occurrence, its complications, and surgical interventions [26–27]. Moreover, a clear association between bacterial count and gastric mucosal inflammation has been exhibited [28–30]. While Sheu et al. [13] showed the effect of higher serological titers of *H. pylori* on eradication failure, Moshkowitz et al. [15] reported that favorable eradication rates were achieved only in patients with low pre-treatment urease activity assessed by UBT. Even if indirect indicators of *H. pylori* colonization density were used in these studies, these findings provide arguments for the role of bacterial density in *H. pylori* eradication. In other previous research, Lai et al. [31] and Shah et al. [32] aimed to investigate the association of pre-treatment *H. pylori* density with bacterial eradication and ulcer healing rates using a similar method to our study. They found a negative correlation between density and eradication rates consistent with our results. All these results argue for choosing the more effective therapy options in patients with severe *H. pylori* colonization density.

In the present study, we could not conduct bacterial culture or drug sensitivity tests to investigate the presence of antibiotic resistance for *H. pylori*. This limitation could prevent a clearer interpretation of our study results and the role of high density. In previous studies on *H. pylori* and *Escherichia coli*, the effect of bacterial density on antibiotic resistance could not be proven [33–34]. We believe that the main reason for treatment failure in patients with high *H. pylori* density can be related simply to the high bacterial load. In addition, although its density significantly decreased after eradication therapy, *H. pylori* still might be detected in only one part of the stomach (corpus or antrum), as revealed by the histopathological follow-up of high-risk malignancy patients. Further studies are needed to explain the relationship between high density colonization and treatment failure. Another limitation is represented by the lack of a sample size calculation before the study.

CONCLUSIONS

Helicobacter pylori colonization severity, assessed by histopathological grading according to the Sydney classification, may predict the usefulness of eradication therapy in pre-treatment assessment. We recommend the use of more effective therapeutical regimens, such as concomitant and levofloxacin-based triple therapy or quadruple therapy for the first-line *H. pylori* eradication treatment in patients with severe bacterial colonization density. Further research with larger sample sizes and various treatment regimens is required to prove the impact of *H. pylori* colonization density on acceptable eradication rates.

Conflicts of interest: None to declare.

Authors' contributions: H.D., K.O., A.U. designed the study. A.T and A.G.K gathered and analyzed the data. A.G. analyzed the biopsy specimens. A.T. and K.O. drafted the paper. M.G. revised the manuscript. All authors critically revised and approved the final version for publication and agreed to be accountable for all aspects of the work.

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