The Influence of Iron Deficiency on *Helicobacter pylori* Eradication

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Background/Aims: *Helicobacter pylori* is a distinctive pathogen that lives in the gastric mucosa and is a well known risk factor of gastric adenocarcinoma. Iron deficiency aggravates the development of *H. pylori*-induced premalignant and malignant lesions in a *cagA*-dependent manner, enhancing *H. pylori* virulence. The aim of this study was to identify the relationship between iron deficiency and *H. pylori* eradication rates.

Materials and Methods: Participants who received 7 days of first-line triple therapy with serum iron level measured in parallel were retrospectively investigated between 2005 and 2014. *H. pylori* eradication was confirmed by the rapid urease test or ¹³C-urea breath test at least 4 weeks after completion of triple therapy. Iron deficiency was defined as either a serum iron level less than 50 μ g/dL or a serum ferritin level less than 12 ng/mL.

Results: A total of 194 patients received 7 days of first-line triple therapy along with parallel serum iron level measurements over the 10-year period. The mean average age was 53.3 years (range, 21~86 years), and 135 patients (69.6%) were male. The overall *H. pylori* eradication rate was 83.5%. Proportions of eradication success with ferritin level less than 12 ng/mL and iron less than 50 μ g/dL were 90.5% and 88.6%, respectively. However, there was no statistical difference in eradication rates according to iron deficiency.

Conclusions: Iron deficiency might not be related with *H. pylori* eradication rates in this study. Further large-scale studies are needed to confirm this result. (Korean J *Helicobacter* Up Gastrointest Res 2016;16:82-87)

Key Words: *Helicobacter pylori*; Disease eradication; Iron

INTRODUCTION

Helicobacter pylori causes a unique bacterial infection of the gastric mucosa and is the main risk factor of gastric adenocarcinoma.¹ The International Agency for Research on Cancer, a subgroup of the World Health Organization (WHO), has declared that *H. pylori* is a definite gastric carcinogen (group I).^{2,3} Therefore, eradication is necessary to maintain public health, particularly in areas with high prevalence, such as Korea.

Iron deficiency is the most common micronutrient deficiency and affects more than 2 billion people worldwide.⁴ Further, iron deficiency is supposed to be one of the risk factors of gastrointestinal tract neoplasms, including the stomach.^{5,6} Interestingly, a recent study demonstrated increased severity of proinflammatory conditions in *H. pylori* infected patients with iron deficiency in comparison to

H. pylori infected patients with normal iron levels, suggesting that iron deficiency increases *H. pylori* virulence.⁷

In terms of *H. pylori* eradication, several established studies have substantiated that poor compliance and antibiotic resistance are related with eradication therapy.^{8,9} In addition, other factors including age, sex, smoking, alcohol, and aspirin use also seem to affect *H. pylori* eradication failure.⁹⁻¹¹ Our study group has reported that female sex and smoking are associated with eradication failure.¹² However, the efficacy of iron deficiency, which could enhance *H. pylori* virulence, relative to eradication is unknown. The present study aimed to verify the relationship between serum iron deficiency and *H. pylori* eradication.

MATERIALS AND METHODS

1. Study population

Participants who visited Kosin University Gospel Hospital between January 2005 and August 2014, were confirmed to have *H. pylori* infection, received a first-line proton

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pump inhibitor (PPI)-based triple therapy, and concurrently measured for iron deficiency were enrolled in our study. *H. pylori* infection was diagnosed using the rapid urease test or the ^{13}C -urea breath test before and after eradication therapy. Participants were excluded if they were lost to follow-up or were poorly compliant. Compliance was classified as good and poor based on pill count. Participants who took $\geq 80\%$ of the prescribed medicine were considered of good compliance, and those who took $< 80\%$ of the prescribed medicine were placed in the poor compliance group. Patients were excluded if they received first-line PPI-based triple therapy for longer than 7 days or another antibiotic-containing eradication therapy (e.g., 1.0 g amoxicillin, 500 mg clarithromycin, and 250 mg metronidazole twice daily) or took iron supplements as reported in the medical records.

We also identified demographic data, smoking and alcohol habits, comorbidities (such as hypertension or diabetes mellitus), area of residence, and endoscopic diagnoses. Urban residence was defined as living in the metropolitan cities of Korea, and rural residence was defined as not living in the metropolitan cities of Korea. Endoscopic features, such as gastric ulcers, duodenal ulcers, gastric and duodenal ulcers, previous endoscopic submucosal dissection (ESD) state due to adenoma or early gastric cancer (EGC), mucosa-associated lymphoid tissue (MALT) lymphoma, gastritis, dyspepsia, and gastric polyps were verified by gastroduodenoscopy or gastroduodenoscopy with or without biopsy. This study protocol was approved by the Institutional Review Board of Kosin University Gospel Hospital (IRB no. 2016- 04-023).

2. Laboratory variables

We investigated the laboratory variables that were associated with anemia and iron deficiency. The variables were hemoglobin (Hb), iron, and ferritin, which were measured before *H. pylori* eradication therapy. Anemia was graded by severity based on WHO criteria and was defined as Hb level less than 10 g/dL, which is moderate anemia based on WHO criteria.¹³ Iron deficiency was defined as either serum iron levels less than 50 $\mu\text{g/dL}$ or serum ferritin levels less than 12 ng/mL.¹⁴

3. *H. pylori* eradication therapy and follow-up

Participants with *H. pylori* infection received eradication therapy comprised of standard-dose PPIs, 1.0 g amoxicillin, and 0.5 g clarithromycin twice daily for 7 days. A rapid urease test or a ^{13}C -urea breath test was performed to confirm eradication at least 4 weeks after treatment completion.

4. Rapid urease test

To ascertain *H. pylori* infection with the rapid urease test (CLO test; Delta West, Bentley, WA, Australia), endoscopic biopsies were acquired from the gastric mucosa. The tissue sample was immersed in the rapid urea reagent. The result was positive if the reagent color changed from yellow to red by 12 hours later. The result was negative if there was no change in reagent color.

5. ^{13}C -urea breath test

Participants fasted for at least 4 hours before the first breath sample was obtained. Afterward, tablets containing 100 mg of ^{13}C -urea (UBiT; Otsuka Pharmaceutical, Tokyo, Japan) were administered to participants with 100 mL water orally. Twenty minutes after ingesting the tablets, the second breath sample was collected. Using the collected breath samples, *H. pylori* infection was analyzed by ^{13}C -urea breath test (UBiT-IR300; Otsuka Electronics, Osaka, Japan). The cut-off value of the current procedure was 2.5‰.

6. Statistical analyses

All statistical analyses were conducted using the PASW Statistics ver. 18.0 (IBM Co., Armonk, NY, USA). *H. pylori* eradication rate was evaluated by per-protocol analysis. Categorical variables were analyzed using a chi-square (χ^2) test, and continuous variables were analyzed using t-test. A *P* value < 0.05 was regarded as statistically significant.

RESULTS

1. Participant characteristics

A total of 194 patients were concurrently checked for

anemia based on serum iron levels and received 7 days of first-line PPI therapy. The average age (mean±SD) was 53.3±15.3 years (range, 21~86 years), and 135 patients (69.6%) were male. A total of 162 out of 194 patients achieved successful eradication by per-protocol analysis. Therefore, the overall *H. pylori* eradication rate was 83.5%. All patients underwent gastroduodenoscopy and were diagnosed with gastric ulcers (71 patients, 36.6%), duodenal ulcers (71 patients, 36.6%), gastric and duodenal ulcers (24 patients, 12.4%), and previous ESD state due to adenoma or EGC (9 patients, 4.6%). Other conditions included MALT lymphoma, gastritis, dyspepsia, and gastric polyps (19 patients, 9.8%). Clinical data and demographic features are described in Table 1.

2. Verification of related factors according to eradication failure

The proportion of iron deficiency was 22.7% (44/194) and 13.0% (21/161) according to serum iron and ferritin levels, respectively. When we evaluated the laboratory findings based on the presence of iron deficiency, the

eradication rates were higher in patients with iron deficiency when compared with patients without iron deficiency. The eradication rates were 90.5% for serum ferritin <12 ng/mL group and 88.6% for serum iron level <50 µg/dL group. Unfortunately, there was no statistical difference between eradication according to Hb, serum iron, or ferritin levels (Table 2). Related factors of erad-

Table 2. Laboratory Factors Related with Eradication Failure of Proton Pump Inhibitor-based Triple Therapy

Parameter	Eradication success (n=162)	Eradication failure (n=32)	P value
Hemoglobin (g/dL)			0.673
≥10	117 (84.2)	22 (15.8)	
<10	45 (81.8)	10 (18.2)	
Ferritin (ng/mL) ^a			0.742
≥12	118 (84.3)	22 (15.7)	
<12	19 (90.5)	2 (9.5)	
Iron (µg/dL)			0.362
≥50	123 (82.0)	27 (18.0)	
<50	39 (88.6)	5 (11.4)	

Values are presented as n (%).

^aSome data are missing and missing values were not included.

Table 1. Baseline Characteristics of the Participants (n=194)

Characteristic	Value
Age (yr)	53.3±15.3
Gender	
Male	135 (69.6)
Female	59 (30.4)
Cigarette smoking	73 (37.6)
Alcohol intake	107 (55.2)
Hypertension	47 (24.2)
Diabetes mellitus	23 (11.9)
Residence	
Rural	30 (15.5)
Urban	164 (84.5)
<i>Helicobacter pylori</i> eradication	
Success	162 (83.5)
Fail	32 (16.5)
Endoscopic diagnosis	
Gastric ulcer	71 (36.6)
Duodenal ulcer	71 (36.6)
Gastric ulcer + duodenal ulcer	24 (12.4)
Post ESD due to adenoma or EGC	9 (4.6)
Others ^a	19 (9.8)

Values are presented as mean±SD or n (%).

ESD, endoscopic submucosal dissection; EGC, early gastric cancer.

^aOthers include mucosa-associated lymphoid tissue lymphoma, gastritis, dyspepsia, and gastric polyp.

Table 3. Clinical Factors Related with Eradication Failure of Proton Pump Inhibitor-based Triple Therapy

Characteristic	Eradication success (n=162)	Eradication failure (n=32)	P value
Age (yr)			0.702
<50	60 (82.2)	13 (17.8)	
≥50	102 (84.3)	19 (15.7)	
Gender			0.594
Male	114 (84.4)	21 (15.6)	
Female	48 (81.4)	11 (18.6)	
Residence			1.000
Rural	25 (83.3)	5 (16.7)	
Urban	137 (83.5)	27 (16.5)	
Cigarette smoking			0.434
No	103 (85.1)	18 (14.9)	
Yes	59 (80.8)	14 (19.2)	
Alcohol intake			0.521
No	71 (81.6)	16 (18.4)	
Yes	91 (85.0)	16 (15.0)	
Hypertension			0.573
No	124 (84.4)	23 (15.6)	
Yes	38 (80.9)	9 (19.1)	
Diabetes mellitus			0.772
No	142 (83.0)	29 (17.0)	
Yes	20 (87.0)	3 (13.0)	

Values are presented as n (%).

ication failure in accordance with clinical features are summarized in Table 3. There were no statistically significant clinical factors associated with eradication failure.

DISCUSSION

It is well known that *H. pylori* is associated with iron deficiency anemia (IDA). There are many causes of IDA including chronic gastrointestinal bleeding, menorrhagia, malabsorption, and vegetarian diet.¹⁵ Among them, *H. pylori* infection is known to induce IDA by reducing iron absorption. The proposed mechanism is that *H. pylori* competes with the host for iron, which decreases the bioavailability of vitamin C and might stimulate micro-erosions that lead to bleeding.¹⁶ *H. pylori* eradication has been shown to be useful in recovering Hb, serum iron, and ferritin levels in IDA patients with infection in meta-analysis studies.^{17,18} The Maastricht IV/Florence Consensus Report recommends that *H. pylori* should be confirmed and eradicated in patients with unexplained IDA.¹⁹

Interestingly, a recent animal and human study approached iron deficiency in connection with *H. pylori* infection from a different perspective.⁷ *H. pylori* infection is the major influencing factor of gastric carcinogenesis, and *H. pylori* with the *cag* pathogenicity island (*cag*⁺) that encodes CagA and a type IV secretion system (T4SS) leads to a worse prognosis as the disease progresses.²⁰ This study accessed iron deficiency, which is one of the environmental factors that can alter the risk of carcinogenesis in the stomach. In this study, the virulence of *H. pylori* increased in iron deficiency conditions, and pathogenesis seemed to be facilitated by increased production and function of the *cag* T4SS.⁷

A meta-analysis reported that eradication rates were significantly higher in patients with *cagA*-positive strains than in *cagA*-negative strains (risk ratio, 2.0; 95% CI, 1.6~2.4; $P<0.001$).²¹ Thus, *cagA* was considered as a predictive factor for successful *H. pylori* eradication. It is presumed that severe gastric inflammation induced by *cagA* enhances mucosal blood flow and antibiotic effects by diffusion of antibiotics.^{22,23} In addition, *cagA*-positive strains grow faster than *cagA*-negative strains and can be influenced more by antibiotics.²⁴ We investigated the rela-

tionship between iron deficiency and *H. pylori* eradication rates using Hb, serum iron, and ferritin, based on the prediction that iron deficiency increases *H. pylori* virulence and affects *H. pylori* eradication. The eradication rates of patients with iron deficiency were higher when compared with patients without iron deficiency in the current study. However, anemia and iron deficiency did not show a statistically significant difference in the eradication success and eradication failure groups. To the best of our knowledge, there have been few studies investigating the effect of iron deficiency for *H. pylori* eradication. Miernyk et al.¹⁴ identified the efficacy of *H. pylori* infection on iron deficiency in native Alaskan adults. In their study, iron deficiency before eradication therapy did not affect *H. pylori* eradication ($P=0.63$) in accordance with our study. Therefore, well-designed, prospective studies are needed to confirm the influence of iron deficiency in *H. pylori* eradication.

As mentioned above, antibiotic resistance such as that to clarithromycin and metronidazole is the main reason for *H. pylori* eradication therapy failure.^{8,9} In terms of host factors, poor compliance is the major factor of *H. pylori* eradication failure.^{8,9} In addition, gene polymorphisms, type of disease, or smoking are considered to be related to *H. pylori* eradication failure.²⁵ On closer scrutiny, PPIs are mainly metabolized via the cytochrome P450 (CYP) 2C19 channel; therefore, genetic polymorphisms of CYP2C19 could influence eradication. Padol et al.²⁶ showed high *H. pylori* eradication rates in a poor metabolizer group compared with a homozygous extensive metabolizer group (OR, 4.03; 95% CI, 1.97~8.28; $P=0.0001$) and the heterozygous extensive metabolizer group (OR, 2.24; 95% CI, 1.09~4.61; $P=0.03$). These results suggest that gene polymorphisms including CYP2C19 affect *H. pylori* eradication failure. A study from France revealed that *H. pylori* eradication rates are low in patients with duodenal ulcers and functional dyspepsia ($P<10^{-6}$), with rates of eradication failure of 21.9% and 33.7%, respectively.²⁷ In addition, it is thought that smoking affects *H. pylori* eradication failure due to decreased gastric blood flow, and the secretion of mucus due to smoking could reduce antibiotic delivery to the gastric mucosa, decreasing the effects of eradication therapy.^{9,28}

Female sex and smoking were associated with eradication failure in a previous study by our group.¹² In this study, there were no statistically significant clinical factors related with eradication failure. We think there is a selective bias in the current study that affected the results; therefore, further prospective studies are required to find other clinical factors that are related with *H. pylori* eradication failure.

There are several limitations to this study. Our study was a retrospective analysis in a single center, and selective bias was present according to patient enrollment. We did not diagnose *H. pylori* by histology before or after eradication therapy. In addition, the proportion of patients with iron deficiency was small. However, studies that have evaluated the relationship between iron deficiency and *H. pylori* eradication are rare, and the conception of the current study is novel. Thus, our results might be helpful for future research in this area.

In conclusion, iron deficiency might not be associated with *H. pylori* eradication in our study. Henceforth, well-designed, prospective studies on a larger scale are needed to demonstrate the effect of iron deficiency on *H. pylori* eradication therapy.

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