

[ORIGINAL ARTICLE]

Relationship of *Helicobacter pylori* Infection with Gastric Black Spots Shown by Endoscopy

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Abstract:

Objective The recently reported endoscopic finding of black spots is defined as black pigmentation in gastric mucosa. We attempted to clarify the relationship between the *Helicobacter pylori* infection status and black spot occurrence.

Methods The study subjects were 1,600 individuals who underwent an annual medical checkup and whose *H. pylori* status could be determined. Upper endoscopic examinations were performed in all, and the presence of black spots in the stomach as well as the degree of gastric mucosal atrophy were determined.

Results Among the 1,600 enrolled subjects, 784 underwent eradication for *H. pylori*, of whom 144 were originally *H. pylori*-positive and 672 *H. pylori*-negative. Black spots in the stomach were observed in 156 (9.8%). The rate of prevalence of black spots in the *H. pylori*-positive and *H. pylori*-negative subjects was 2.1% and 1.5%, respectively, while that in subjects after undergoing eradication of *H. pylori* was 18.2%. A multiple logistic regression analysis demonstrated that an older age and post-eradication status were significant factors for black spot occurrence, while proton pump inhibitor treatment showed a tendency to be a risk factor. In subjects with post-eradication status, a higher grade of gastric mucosal atrophy was a significant risk factor for the occurrence of black spots.

Conclusion *H. pylori* post-eradication status and an older age were significant factors related to the appearance of black spots, and a higher grade of gastric mucosal atrophy was also a significant risk factor in subjects who had undergone successful eradication.

Key words: black spots, stomach, endoscopy, *Helicobacter pylori*, eradication

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Introduction

The recently reported endoscopic finding known as “black spots” is defined as black pigmentation in the gastric mucosa on conventional endoscopy (1). A histological study using autopsy and endoscopical biopsy samples showed that these black spots are composed of brownish pigmentation, including tiny birefringent crystals and eosinophilic materials in a dilated fundic gland, although neither the precise components nor the mechanisms related to their occurrence have been clarified (1). Thus, these black spots are completely different from hematin attached to the gastric mucosal surface.

Such black spots are only observed in the fundic gland region and are often seen in subjects who have undergone proton pump inhibitor (PPI) or corticosteroid administration (1). We have also frequently noted endoscopic findings of black spots in the fundic gland area of subjects who have undergone successful *Helicobacter pylori* eradication. Black spots on endoscopy might be a characteristic finding after successful eradication, so the present study was performed to clarify the relationship between the *H. pylori* infection status and the occurrence of gastric black spots in individuals who visited our medical center for an annual comprehensive medical checkup.

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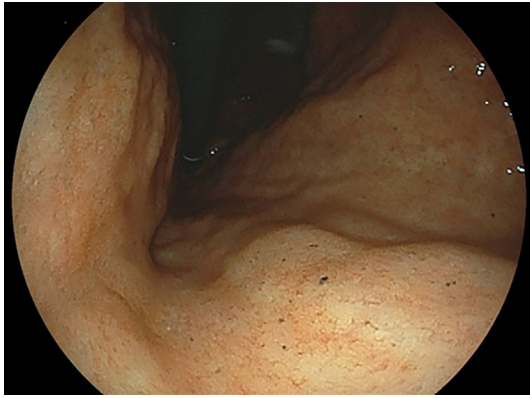


Figure 1. Representative case. A 68-year-old man who underwent eradication therapy for *H. pylori* 5 years prior to the examination. Endoscopy revealed multiple black spots in the body of the stomach.

Materials and Methods

The study subjects consisted of individuals who visited the Health Center of Shimane Environment and Health Public Corporation for an annual medical checkup examination between September 2017 and March 2018. The majority were socially active and productive and considered to be socioeconomically middle class. Those with a history of gastric surgery were excluded. For this study, we selected patients in whom the status of *H. pylori* infection could be determined by medical records and/or serum antibody test findings and then examined the relationship between the prevalence of black spots and the infection status.

Serum anti-*H. pylori* IgG antibody detection was performed using a SphereLight *H. pylori* antibody J[®] kit (Wako Pure Chemical Industries, Osaka) (2). The antibody titer was automatically determined using a chemiluminescent enzyme immunoassay method, and a value ≥ 4.0 U/mL was defined as positive, according to the manufacturer's instructions. In addition, a precise medical history concerning the status of *H. pylori* infection (negative, positive, post-eradication) was obtained in an interview with the patient conducted by a public health nurse. The use of medications, including PPIs, within the preceding 12 months was also noted, although the precise history of medications beyond 12 months ago could not be determined. Those without successful eradication were included in the group with *H. pylori* infection even though they had undergone eradication therapy. When eradication therapy was confirmed as not successful, we recommended that the patient take an *H. pylori* stool antigen test at our institution. The presence or absence of *H. pylori* infection and successful eradication were also confirmed based on endoscopic findings obtained in an upper gastrointestinal (GI) endoscopic examination (3-7), which was performed for all of the subjects.

For the present study, cases with endoscopic gastric mucosal atrophy classified as C2-O3 using the classification of

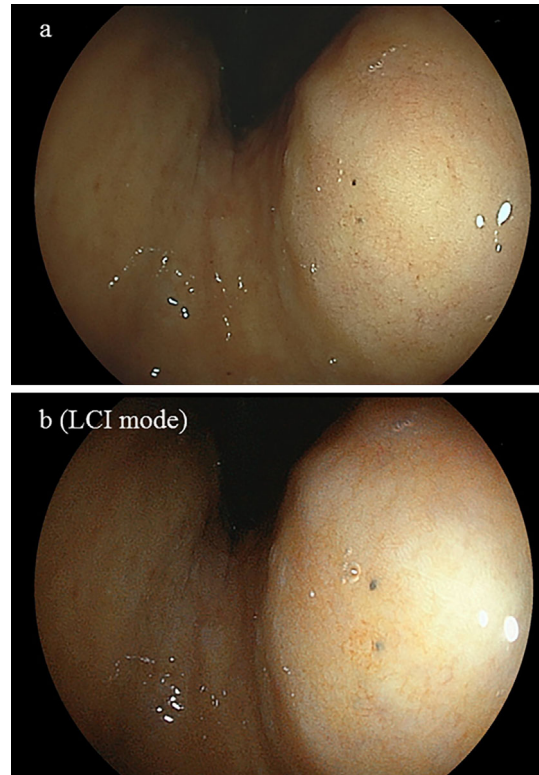


Figure 2. Representative case. A 44-year-old woman who underwent eradication therapy for *H. pylori* 2 years prior to the examination. Endoscopic images showing multiple black spots in the body of the stomach (a: conventional mode, b: LCI mode). Image-enhanced endoscopy with LCI is useful for diagnosing black spots inside the gastric gland.

Kimura and Takemoto (8) and negative anti-*H. pylori* IgG antibody test findings were excluded, as those might have had a post-eradication status even though they did not previously undergo eradication therapy or were positive for *H. pylori* infection (2, 9). As a result, the present study cohort comprised 1,600 individuals (1,006 men and 594 women; mean age 54.6 ± 9.4 years) in whom the status of *H. pylori* infection was determined.

All upper endoscopic examinations were performed by licensed experienced endoscopists using an EG-L580NW endoscope (Fujifilm, Tokyo, Japan). Black spots in the stomach are usually detected in multiple parts of the body and fornix by conventional endoscopy (Fig. 1). As black spots exist inside the gastric gland, they can be distinguished from hematin attachment at the gastric mucosal surface. Although the presence of black spots can be easily identified on conventional imaging, image-enhanced endoscopy with the linked color imaging (LCI) mode (10) is useful for diagnosing black spots inside the gastric gland (Fig. 2, 3). For the present study, positivity for black spots was determined when such lesions were observed in the stomach, even if study subjects had only a single spot. The degree of gastric mucosal atrophy was evaluated and divided into six categories (C1, C2, C3, O1, O2, O3, in order) using the classification of Kimura and Takemoto (8). For this study, we defined

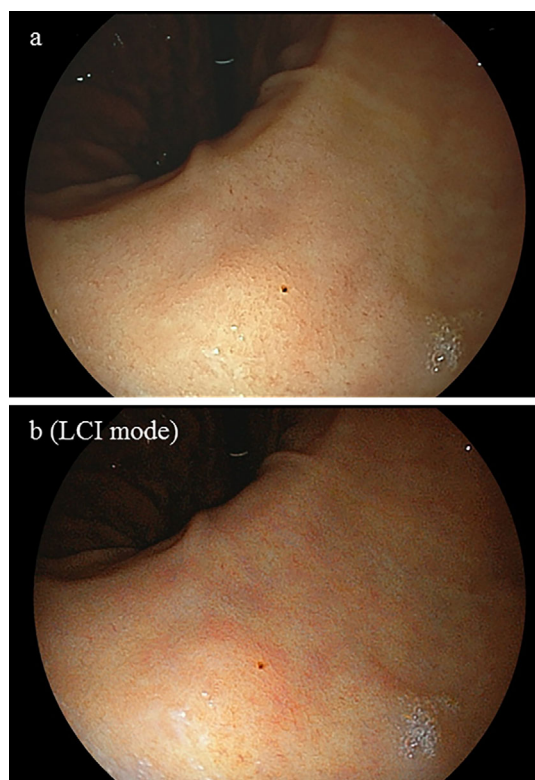


Figure 3. Representative case. A 63-year-old man without *H. pylori* infection. Endoscopic images showing hematin on the antral mucosa of the stomach (a: conventional mode, b: LCI mode). Endoscopic imaging with LCI was able to easily distinguish between black spots and hematin attached to the gastric mucosa.

C1-C2 as mild, C3-O1 as moderate, and O2-O3 as severe gastric mucosal atrophy. All endoscopic images obtained from each subject were simultaneously reviewed by three licensed endoscopists, and a diagnosis of each endoscopic finding was made. If there were any inconsistencies in the reading of the endoscopic images among the endoscopists, the final diagnosis was decided by one of the endoscopists (K.A.).

Statistical analyses were performed using a chi-squared test and Mann-Whitney's U test. A multiple logistic regression analysis was also performed to calculate the odds ratios after adjusting for confounding factors. The Stat View software program, ver. 5.0 (Abacus Concepts, Berkeley, USA) for Macintosh, was used to perform the statistical analyses. P values <0.05 were considered to be statistically significant.

This study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Shimane Institute of Health Science. Written informed consent, indicating that clinical data without the release of individual information would be used for a clinical study, was obtained from all of the subjects prior to the medical checkup examinations.

Table 1. Characteristics of Subjects with and without Endoscopic Findings of Black Spots.

	Black spots		p value
	Positive	Negative	
Number of subjects	156	1444	
Gender (male/female)	105/51	901/543	0.228
Age (years)	58.3±0.7	54.2±0.2	<0.001
PPI user	14 (9.0)	71 (4.9)	0.032
<i>H. pylori</i> infection			<0.001
Negative*	10 (1.5)	662 (98.5)	
Positive*	3 (2.1)	141 (97.9)	
Post-eradication	143 (18.2)	641 (81.8)	

Data are expressed as the mean±SE or number of subjects. Values in parentheses indicate percentage. BMI: body mass index. *Significantly different in comparison with post-eradicated subjects.

Results

Of the 1,600 study subjects, 784 had undergone eradication for *H. pylori*, of whom 144 were originally positive for *H. pylori* and 672 were originally negative. Black spots were observed in 156 subjects (9.8%) and were found in the area of the fornix and body of the stomach in all cases. The characteristics of subjects with and without such lesions are shown in Table 1. Those with lesions were predominantly older and had typically been administered PPIs compared to those without. The prevalence rates of black spots in *H. pylori*-positive and *H. pylori*-negative subjects were 2.1% and 1.5%, respectively, while that in subjects after undergoing eradication for *H. pylori* was 18.2%. Therefore, the lesions were more frequently observed in subjects who had undergone successful eradication of *H. pylori* than in those with an *H. pylori*-positive or *H. pylori*-negative status. Of all subjects with black spots, 91.7% had an *H. pylori* post-eradication status.

We had access to endoscopic images obtained before undergoing eradication in 109 of 143 cases with black spots and *H. pylori* post-eradication status. In those cases, no endoscopic findings indicating black spots were observed prior to eradication, although the quality of the endoscopic images before eradication was lower than that of those obtained during the study period in many cases. A multiple logistic regression analysis demonstrated that an older age and post-eradication status were significant factors for the occurrence of black spots (Table 2). When an analysis was performed only for subjects with post-eradication status, a higher grade of gastric mucosal atrophy was shown to be a significant risk factor for the occurrence of black spots, while a history of PPI administration showed a tendency to be a risk factor for the occurrence of black spots in subjects with post-eradication status (Table 3, 4).

Table 2. Multiple Logistic Regression Analysis of Endoscopic Finding of Black Spots.

	Odds ratio	95%CI	p value
Gender (male)	1.015	0.700-1.471	0.937
Age (1-year increments)	1.025	1.005-1.045	0.014
PPI use*	1.428	0.754-2.704	0.275
<i>H. pylori</i> infection (vs. negative)			
Positive	1.361	0.369-5.015	0.644
Post-eradication	13.153	5.845-18.053	<0.001

*Present PPI use

Table 3. Characteristics of Subjects with and without Endoscopic Findings of Black Spots Following Eradication of *H. Pylori*.

	Black spots		p value
	Positive	Negative	
Number of subjects	143	641	
Gender (male/female)	96/47	424/217	
Age (years)	58.2±0.8	56.6±0.4	0.073
PPI user	13 (9.1)	38 (5.9)	0.165
Gastric mucosal atrophy			<0.001
Mild	40 (10.7)	70 (89.3)	
Moderate*	75 (24.0)	238 (76.0)	
Severe*	28 (28.6)	70 (71.4)	

Data are expressed as the mean±SE or number of subjects. Values in parentheses indicate percentage. BMI: body mass index. *Significantly different in comparison with mild atrophy.

Discussion

In the present study, we investigated the prevalence of black spots in the stomach of subjects who visited our center for an annual medical checkup. The endoscopic finding of black spots is reported to only be observed in the fundic gland region and is often noted in subjects who have been administered PPIs and corticosteroids (1). However, the relationship between the prevalence of black spots and the status of *H. pylori* infection has not been investigated. Our results clearly showed that black spots frequently occurred in subjects who had previously undergone *H. pylori* eradication therapy. In addition, a higher grade of gastric mucosal atrophy was found to be a significant risk factor for the prevalence of black spots, while PPI administration showed a tendency to be a risk factor.

A previous report noted that histological studies of these lesions failed to clarify their precise components (1), and the present findings do not explain why these black spots develop after eradication of *H. pylori*. However, some changes in gastric mucosa following successful eradication therapy are considered to occur and may play an important role in the formation of these spots. As gastric acid secretion has been shown to increase after *H. pylori* eradication, especially in subjects with a higher degree of gastric mucosal at-

rophy (11), the increased gastric acid secretion following therapy for eradication of *H. pylori* may correlate with the formation of black spots. In addition, gastric mucosal atrophy has been shown to improve after eradication for *H. pylori* (12-14), and hypergastrinemia induced by PPI administration is demonstrated to cause hyperplasia of fundic gland (15-18). Therefore, several mechanisms involving the stimulation of the regeneration or hyperplasia of the fundic gland mucosa may be related to the occurrence of black spots. Additional studies are needed to clarify the mechanism underlying the development and precise components of black spots in the stomach.

Several studies have reported changes in endoscopic findings after the successful eradication of *H. pylori* infection, including significantly reduced gastric juice non-transparency, diffuse redness of fundic mucosa, enlarged folds, spotty redness of fundic mucosa, and flat erosions in the stomach compared to cases of failed eradication (3-6). Although such endoscopic findings are considered to disappear or decrease after eradication, their frequency before and after eradication therapy has not been clarified. Map-like redness is a characteristic endoscopic finding in cases of successful *H. pylori* eradication (3-6), although it is not observed in all cases after eradication, and its rate of prevalence has not been clarified. We recently noted that endoscopic findings of multiple white and flat elevated lesions were frequently observed in subjects after successful eradication of *H. pylori*, although they were also observed in subjects without such an infection (19). In that study, multiple white and flat elevated lesions were observed in 51 (4.6%) of 1,003 cases confirmed to have successful *H. pylori* eradication and only 2 (0.5%) of 368 positive for infection, indicating that endoscopic evidence of multiple white and flat elevated lesions is important for confirming that eradication therapy was successful. Another study reported that 48% of cases with black spots had multiple white and flat elevated lesions in the stomach (1). Thus, both black spots and multiple white and flat elevated lesions are considered to reflect a similar condition of the stomach. Indeed, aging, a higher grade of gastric mucosal atrophy, and the administration of an anti-secretory agent have been shown to be risk factors for the occurrence of multiple white and flat elevated lesions (19-21).

In the present study, the rates of prevalence of black spots

Table 4. Multiple Logistic Regression Analysis of Endoscopic Finding of Black Spots in *H. Pylori*-eradicated Subjects.

	Odds ratio	95%CI	p value
Gender (female)	1.066	0.713-1.594	0.754
Age (1-year increments)	1.003	0.981-1.026	0.791
PPI use	1.588	0.801-3.148	0.186
Gastric mucosal atrophy (vs. mild)			
Moderate	2.642	1.725-4.045	<0.001
Severe	3.205	1.783-5.763	<0.001

shown by endoscopy in *H. pylori*-positive and *H. pylori*-negative subjects were 2.1% and 1.5%, respectively, while that in subjects after undergoing *H. pylori* eradication was 18.2%. In addition, those lesions were not detected before the performance of eradication therapy in cases with black spots observed after successful eradication. Therefore, the endoscopic finding of black spots is considered to be important for demonstrating the success of eradication therapy and is observed more often in cases with successful eradication than are multiple white and flat elevated lesions. Nevertheless, further studies are needed to clarify the significance of endoscopic findings such as map-like redness and multiple white and flat elevated lesions, as well as black spots, in individuals who have undergone successful eradication.

Our study is limited by its cross-sectional nature. In addition, the six-month period of observation was short and did not allow us to investigate the time-course endoscopic changes for the prevalence of black spots. A time-course endoscopic study should be performed to clarify the relationship between the duration after eradication of *H. pylori* and the black spot prevalence. In addition, the majority of our study subjects were healthy individuals who visited a medical center for an annual medical checkup examination, and their precise history of medications could not be clarified in the medical records. Thus, the number of patients who had been administered drugs was small, and the effects of medications aside from PPIs on the prevalence of black spots could not be determined. An additional large-scale study is needed in order to clarify the influence of drug administration on the occurrence of black spots.

In conclusion, *H. pylori* post-eradication status and an older age were significant factors related to the appearance of black spots, and a higher grade of gastric mucosal atrophy was also a significant risk factor in subjects who had undergone successful eradication.

The authors state that they have no Conflict of Interest (COI).

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