Original Article

Intragastric bacterial infection and endoscopic findings in *Helicobacter pylori*-negative patients

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In the present study, the authors examined the association between gastric bacterial infection and gastric endoscopic findings in *Helicobacter pylori* (*H. pylori*)-negative patients. The subjects were 105 *H. pylori*-negative patients. The mean age was 72.8 ± 9.1 years. Endoscopy and gastric juice culture were performed. The presence or absence of endoscopic findings was checked according to the Kyoto classification of gastritis. Culture was positive in 69 patients (65.7%), with *Streptococcus* α-hemolytic being the most common (51 patients), followed by *Neisseria* sp. (43 patients). According to the univariate analysis, there was a significant difference between the results of culture and background factors in the use of gastric antisecretory drugs and between the results of culture and various endoscopic findings in atrophic gastritis, intestinal metaplasia, regular arrangement of collecting venule, mucosal swelling, sticky mucus, hyperplastic polyps, hematin, and gastric cobblestone-like lesions. Furthermore, multivariate analysis revealed significant differences in background factors such as the use of gastric antisecretory drugs and endoscopic findings only in patients with mucosal swelling. Endoscopic findings of non-*H. pylori* bacteria-positive gastritis differed from endoscopic findings of *H. pylori*-infected gastritis in several respects. In conclusion, our results suggest that non-*H. pylori* bacteria may infect the stomach and cause gastric inflammation, especially in patients who long term use gastric antisecretory drugs.

***Key Words*:** **intragastric bacterial infection, proton pump inhibitors, gastric antisecretory drugs, Kyoto classification of gastritis, non-*H. pylori* infection**

The stomach is an acidic environment where bacteria cannot live, but Dr. Warren and Dr. Marshall reported in the Lancet in 1983 that *Helicobacter pylori* (*H. pylori*) can infect the stomach.[1] *H. pylori* mainly infects the gastric mucosa during childhood. *H. pylori* infection in the stomach during childhood causes gastritis in all patients, followed by persistent infection and active gastritis, which progresses from gastritis, such as nodular gastritis, to atrophic gastritis and intestinal metaplasia. According to Correa's hypothesis, pathological and epidemiological studies have shown that *H. pylori* infection of the normal gastric mucosa leads to a multistep process of chronic gastritis, atrophy, intestinal metaplasia, and dysplasia. A hypothesis regarding a human model of carcinogenesis has been reported.[2]

In recent years, there have been remarkable advances in research methods in the field of bacteriology. As new molecular biological methods such as 16S rRNA gene sequencing using next-generation sequencing have enabled detailed identification of bacteria in the gastric mucosa, researchers have made new reports regarding the role of non-*H. pylori* bacteria in the development of gastric cancer. In the past, only *H. pylori* infecting the gastric mucosa was thought to be involved in the transition from normal gastric mucosa to gastric carcinogenesis. Stewart *et al.*[3] reported that the amount of *H. pylori* in the gastric mucosa increases after initial infection, especially during the stage of active gastritis. Furthermore, it was reported that the bacterial amount of *H. pylori* is much lower in gastric cancer patients than in patients with gastritis.[4] This phenomenon can be explained by the fact that after *H. pylori* infection, inflammation persists, and acid-secreting parietal cells disappear, making the gastric environment favorable for the formation of other bacterial colonies and accelerating the progression of chronic gastritis to atrophic gastritis and intestinal metaplasia.[5] In addition, it was reported that commensal oral or intestinal microorganisms become enriched as chronic gastritis progresses to atrophic gastritis and intestinal metaplasia.[6,7] In the later stages of gastric precancerous lesions, the gastric environment is no longer suitable for *H. pylori*, and the amount of *H. pylori* decreases. This phenomenon has been confirmed in human studies.[4,5]

Hirschl *et al.*[8–10] reported that *H. pylori* is present in the gastric mucosa of more than 90% of patients with active acute gastritis, but in the majority of patients with advanced atrophic gastritis, intestinal metaplasia or gastric cancer, *H. pylori* is absent in the gastric mucosa even when the anti-*H. pylori* antibody test is positive. Karnes *et al.*[11] reported that *H. pylori* infection disappears in advanced stages of gastric carcinogenesis. At present, the detailed correlation between the diversity of the gastric microbiota and the progression from normal gastric mucosa to gastric cancer has not yet been clarified. Furthermore, there is currently no consensus on the relationship between the diversity of the microbiota and the stage of gastric cancer development. However, it is plausible that an increase or decrease in the diversity of the microbiota in the stomach is associated with the development of gastric cancer.

H. pylori eradication is widely and aggressively practiced worldwide, and in Japan, there were more than 1.6 million cases per year since endoscopic gastritis was covered by insurance in February 2013.[12] *H. pylori* eradication improves gastric acid secretory capacity, and the stomach is thought to be free of bacteria, including *H. pylori*.[13] However, recent analyses of microbial sequences revealed distinct microbial clusters reflecting an increase in bacterial diversity ($p<0.00001$) after 1 year of eradication, indicating that gastric bacteria, mainly oral bacteria, are involved in the progression of gastric carcinogenesis after *H. pylori* eradication.[14]

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The *H. pylori* infection rate was more than 70% in the 1970s but rapidly declined to the 20% range in the 2000s.[15] In 2018, the annual infection rate among 35-year-olds was reported to be less than 1 in 10, with 10.1% for males and 9.2% for females.[16] The number of *H. pylori*-negative patients is clearly increasing, as *H. pylori* eradication is also progressing. Nishizawa *et al.*[17] reported a increase in *H. pylori* uninfected gastric cancer in recent years. This is also accompanied by an increase in reflux esophagitis, an acid-related disease, as well as the prescription of low-dose aspirin for secondary prevention of cerebral and myocardial infarction and NSAIDs for knee arthritis and lumbago due to the aging of the population. Proton pump inhibitors (PPIs) or histamine 2-receptor antagonists (H2As) are recommended in guidelines for the initial and maintenance treatment of reflux esophagitis and for the prevention of aspirin and NSAID ulcers, and their long-term use is increasing.[18,19] Since their marketing, there have been concerns about the effects of PPIs on the development of gastric cancer due to their strong inhibition of gastric acid secretion.[20] The acidic environment of the stomach is the first line of defense against orally ingested bacteria. When the intragastric pH is greater than 4, bacterial colony formation becomes possible in the stomach, and the positivity rate of non-*H. pylori* bacteria in the gastric juice increase, and they settle in the gastric mucosa in long-term use of PPIs.[21]

Nagata *et al.* constructed a large database integrating lifestyle and clinical information with the gut microbiome by shotgun metagenomic sequencing of fecal samples from approximately 4,000 Japanese subjects and comprehensively investigated factors affecting the gut microbiome and reported that long-term PPI administration had the greatest impact on the gut microbiome.[22] It is possible that gastrointestinal bacterial infections other than *H. pylori*, mainly oral bacteria, occur in the stomach. In this study, we performed gastric juice culture tests during endoscopy in *H. pylori*-negative patients. We also investigated the status of non-*H. pylori* bacteria and endoscopic gastritis findings. We also examined the relationship between gastric juice culture and lifestyle habits, as well as the use of gastric antisecretory drugs.

Methods

Participants. This retrospective study was conducted at Tokyo Medical University Hospital to investigate the gastric microflora and gastrointestinal endoscopic findings. The study protocol adhered to the ethical principles of the Declaration of Helsinki and was approved by the institutional review board of Tokyo Medical University (T2023-0134). Because this study was retrospective in design and written informed consent was not obtained from each enrolled patient, a document describing an opt-out policy through which potential patients and/or relatives could refuse inclusion was uploaded to the Tokyo Medical University Hospital website.

The subjects were 105 patients who underwent upper endoscopy for upper gastrointestinal screening and surveillance between May 1, 2023, and September 20, 2022. The mean age was 72.8 ± 9.1 years, and the male-to-female ratio was 1.23:1. The physicians performing the endoscopies were specialists of the Japan Gastroenterological Endoscopy Society and excluded patients younger than 20 years, older than 90 years, who were taking antimicrobial agents, and who had undergone gastric resection. The scope used in this study was the GIF-1200 N (Olympus Medical Systems Corporation, Tokyo, Japan). The optical source was an EVIS X1 system (Olympus Medical Systems).

Endoscopy and gastric juice culture. Gastric juice collection: After an endoscope was inserted into the stomach, approximately 10 ml of gastric juice containing gastric mucus was collected from the forceps channel. Blood agar (Trypticase Soy Agar with 5% Sheep Blood) and chocolate II agar (Chocolate II Agar, BD, Fukushima, Japan) were used as culture media. After the media were added to the specimens, both media were incubated with carbon dioxide at 35°C overnight for 24 h. The medium was observed to identify the bacteria that developed. The chocolate medium was then incubated with carbon dioxide at 35°C for another 24 h, the blood agar medium was incubated at aerobic room temperature, and the medium was observed and analyzed again.

The presence or absence of *H. pylori* infection was checked by the *H. pylori* antibody test and ^{13}C-urea breath test.

Endoscopic findings. Endoscopic findings were evaluated using the Kyoto classification of gastritis.[23] To assess the presence of endoscopic gastritis, atrophy, intestinal metaplasia, enlarged folds, tortuous folds, nodularity, diffuse redness, regular arrangement of collecting venules (RACs), fundic gland polyps, map-like redness, mucosal swelling, sticky mucus, spotty redness, foveolar-hyperplastic polyps, xanthomas, hematin, red streaks, raised erosions, multiple white and flat elevated lesions, patchy redness, depressed erosions, body erosions, sticky adherent dense mucus, and gastric cobblestone-like lesions were evaluated. Atrophy, intestinal metaplasia, enlarged folds, tortuous folds, nodularity, and diffuse redness were scored using the Kyoto classification of gastritis.

Statistical analysis. Age and Kyoto classification of gastritis scores are expressed as the mean ± SD. Comparisons between positive and negative results for each factor and among different dosed drugs (no drug, on demand, H2As, PPIs, and P-CAB) were analyzed by *t* tests and one-way ANOVA, respectively. Categorized variables such as sex, smoking status, alcohol consumption status, *H. pylori* status, culture status, and any endoscopic findings are expressed as *n* numbers, and contingency table analysis (*chi*-square test) was used to examine differences between positive and negative factors and among different doses of drugs. Multivariate logistic regression analyses were used to test the associations of candidate variables with positive culture results using gastric juice. $P<0.05$ was considered to indicate statistical significance, and all *p* values were two-sided. Calculations were conducted using SPSS ver. 28 (IBM, Inc., Armonk, NY).

Results

Patient background. The ratio of patients who did and did not smoke was 4:101, and the ratio of patients who did and did not drink was 33:72. For *H. pylori* infection, the ratio of previously infected to uninfected individuals was 82:23. The ratio of patients with and without gastric antisecretory drug use was 55:50, which is almost equal. The H2A/PPI/P-CAB ratio was 3:33:19. PPIs and P-CABs were prescribed for at least 1 year. Four of the PPIs and 4 of the P-CABs were taken on so-called on-demand.

Gastric fluid culture results. Sixty-nine (65.7%) of the cultures were positive for *Streptococcus*-α-hemolytic bacteria, the largest number (51), followed by *Neisseria* sp. (41) and *Streptococcus* sp. (33). Group c included *Streptococcus* (3 patients), *Staphylococuus* aureus (10 patients), *Staphylococuus* epdermidis (1 patient), *Rothia* sp. (1 patient), *Yeast* sp. (4 patients), *Enterobacter aerogenes* (CRE) (1 patient), *Enterococcus faecalis* (2 patients), *Escherichia coli* (3 patients), *Haemophilus parainfluenzae* (5 patients), *Haemophilus parahamolyticus* (1 patient), *Haemophilus hamolyticus* (2 patients), *Bacillus subtilis* (7 patients), *Bacillus cereus* (1 patient), *Bacillus* sp. (7 patients), *Corynebacterium* sp. (3 patients), *Candida albicans* (5 patients), *Candida glabrata* (1 patient), and *Acinetobacter baumanii* (1 patient).

No significant differences were found in the relationships between culture positivity/negativity and smoking status, alcohol consumption, or past *H. pylori* infection, and a significant associ-

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ation was found between the use of gastric antisecretory drugs and no gastric antisecretory drugs, on demand, or continuous daily oral administration. However, no differences were found between H2A, PPI and P-CAB (Table 1).

Endoscopic findings. The relationships between the culture results and various endoscopic findings are shown in Table 2. Positive culture results were significantly associated with atrophic gastritis, intestinal metaplasia, mucosal swelling, sticky mucus, hyperplastic polyps, sticky adherent dense mucus, and gastric cobblestone-like lesions. On the other hand, significant associations were found for culture negativity, RAC, and hematin.

According to the univariate analysis, there was a significant difference between the results of gastric juice culture and background factors in the use of gastric antisecretory drugs and between the results of gastric juice culture and various endoscopic findings in atrophic gastritis, intestinal metaplasia, RAC, mucosal swelling, sticky mucus, hyperplastic polyps, hematin, and gastric cobblestone-like lesions (Table 3 and 4). Furthermore, multivariate analysis revealed significant differences in background factors, such as the use of gastric acid secretion inhibitors and endoscopic findings, but only in mucosal swelling (Table 3 and 4).

Table 1. Characteristics of patients

Factors		
Patients number		105
Sex	male/female	58/47
Age	(year ± SD)	73.9 ± 9.1
Smoking	–/+	101/4
Alcohol	–/+	72/33
H. pylori infection	Never infection/Previous infection	23/82
Acid inhibitor	–/+	50/55
Essential use	H2RA/PPI/P-CAB	3/28/16

Discussion

Over the past decade, we have come to understand the critical

Table 2. Factors associatied wit culture test positivity

Factors		Positive	Negative	*p* value
Patients number		69	36	
Sex	male/female	36/33	22/14	0.382
Age	(year ± SD)	73.3 ± 9.8	71.8 ± 7.6	0.409
Smoking	–/+	67/2	34/2	0.500
Alcohol	–/+	51/18	21/15	0.103
H. pylori infection	Never infection/Previous infection	15/54	8/28	0.955
Acid inhibitor	–/+	20/49	30/6	<0.001
	–/on demand/essential use	20/3/46	30/5/1	<0.001
Essential use	H2A/PPI/P-CAB	3/27/16	0/1/0	0.707
Endoscopy				
Kyoto classification of gastritis	Atrophy	1.45 ± 0.76	1.08 ± 0.69	0.017
	Intestinal metaplasia	1.35 ± 0.84	0.97 ± 0.77	0.027
	Enlarged fold	0.01 ± 0.12	0.00 ± 0.00	0.473
	Nodularity	0.00 ± 0.00	0.00 ± 0.00	—
	Diffuse redness	0.00 ± 0.17	0.00 ± 0.00	0.307
	Total score	2.83 ± 1.55	2.03 ± 1.32	0.010
RAC	–/+	42/27	10/26	0.001
Fundic gland polyp	–/+	50/19	26/10	0.979
Reddished fundic gland polyp	–/+	17/2	10/0	0.567
Map-like redness	–/+	63/6	29/7	0.112
Edematous mucosa	–/+	21/48	32/4	<0.001
Spotty redness	–/+	68/1	36/0	0.468
Sticky mucous	–/+	44/25	35/1	<0.001
Visible vascular pattern	–/+	66/3	36/0	0.204
Hyperplastic polyp	–/+	42/27	29/7	0.041
Xanthoma	–/+	61/8	33/3	0.605
Hematin	–/+	62/7	25/11	0.008
Red streak	–/+	67/2	35/1	0.972
Raised erision	–/+	64/5	32/4	0.697
Multiple white and flat elevated lesions	–/+	55/14	27/9	0.754
Pathy redness	–/+	67/2	36/0	0.302
Depressed erosion	–/+	63/6	31/5	0.523
Erosion in body	–/+	69/0	33/3	0.051
Sticky adherent dense mucus	–/+	57/12	36/0	0.008
Cobblestone-like appearance	–/+	56/13	35/1	0.022

Table 3. Univariate and Multivariate analysis for positive of culture test with reference to background

Factors		Univariate			Multivariate		
		OR	95% CI	*p* value	OR	95% CI	*p* value
Sex	Female	1.44	0.635–3.270	0.383			
Age	(year ± SD)	1.019	0.975–1.065	0.406			
Smoking	+	0.507	0.068–3.761	0.507			
Alcohol	+	0.494	0.211–1.160	0.105			
H. pylori infection	Never infection/Previous infection	0.972	0.368–2.570	0.955			
Acid inhibitor	+ (including on demand + essential)	12.25	4.421–33.944	<0.001	4.600	1.209–17.496	0.035

Table 4. Univariate and Multivariate analysis for positive of culture test with reference to endoscopic findings

Factors		Univariate			Multivariate		
		OR	95% CI	*p* value	OR	95% CI	*p* value
Endoscopy							
Kyoto classification of gastritis	Atrophy	1.911	1.108–3.298	0.020	2.904	0.849–9.929	0.089
	Intestinal metaplasia	1.729	1.056–2.831	0.030	1.021	0.349–2.985	0.97
	Enlarged fold	—					
	Nodularity	—					
	Diffuse redness	—					
	Total score	1.420	1.080–1.867	0.012			
RAC	–/+	0.247	0.103–0.593	0.002	1.545	0.396–6.027	0.531
Fundic gland polyp	–/+	0.988	0.401–2.431	0.979			
Map-like redness	–/+	0.395	0.122–1.279	0.121			
Edematous mucosa	–/+	17.524	5.491–55.930	<0.001	7.125	1,638–30.986	0.009
Spotty redness	–/+	—					
Sticky mucous	–/+	19.855	2.567–154.087	0.004	3.108	0.267–36.196	0.365
Visible vascular pattern	–/+	—					
Hyperplastic polyp	–/+	2.633	1.023–6.932	0.045	1.303	0.302–5.614	0.722
Xanthoma	–/+	1.433	0.358–5.808	0.606			
Hematin	–/+	0.257	0.089–0.737	0.012	0.707	0.189–2.655	0.608
Red streak	–/+	1.045	0.092–11.927	0.972			
Raised erision	–/+	0.500	0.117–2.130	0.349			
Multiple white and flat elevated lesions	–/+	0.764	0.294–1.986	0.58			
Pathy redness	–/+	—					
Depressed erosion	–/+	0.590	0.167–2.086	0.413			
Erosion in body	–/+	0.826	0.152–4.498	0.825			
Sticky adherent dense mucus	–/+	—					
Cobblestone-like appearance	–/+	8.125	1.018–64.863	0.048	2.570	0.208–31.767	0.462

role of microorganisms in the maintenance of health and disease. Biome science, or the study of microbial communities, has grown exponentially in this short period of time. The intestinal microenvironment is composed of microorganisms, the intestinal epithelium, and the local immune system. Microorganisms inhabiting the human gastrointestinal tract include not only bacteria but also viruses and fungi. Diet, breastfeeding, environmental exposure, and genetic factors determine how these microbes develop, and external factors such as antibiotics, mode of birth, and diet affect the microbiome, which appears to be most affected in childhood.[24,25] What is important is the relative abundance and diversity of the microbiome, not just the specific microbes that inhabit a particular environment.[26] The oral microbiome has been implicated in both local disease states, such as dental caries, periodontitis, and oral cancer, and more distant diseases, such as inflammatory bowel disease, pancreatic cancer, and obesity.[27] Although microbiome research has focused on the colon, it is important to understand that other parts of the gut inhabited by microbiota that influence metabolism are also present, namely, the oral cavity and small intestine.[28,29] Compared to those in the large intestine, the absolute (several orders of magnitude) and relative abundances of microorganisms are much lower in the oral cavity and small intestine.

In this gastric juice culture, most of the species detected were *Streptococcus* spp. and *Neisseria* spp. This result is similar to that of Furuta *et al.*,[30] and it seems safe to assume that gastric juice culture and gastric mucosa culture yield similar results. Furthermore, Zilberstein *et al.*[31] reported that the oral flora was dominated by *Streptococcus* and *Neisseria* species, and many of the bacteria detected in the stomach in this study were thought to be of oral origin. Furthermore, PPIs, P-CABs, and other gastric antisecretory drugs were significantly associated with positive gastric juice culture findings in both univariate and multivariate analyses. These results suggest that in long-term patients taking strong gastric antisecretory drugs, an intragastric pH of 4 or higher facilitates the formation of bacterial colonies in the

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stomach, and non-*H. pylori* bacteria, such as oral bacteria, may proliferate and infect the gastric mucosa.

Gastritis has been used as a generic term for symptomatic gastritis, endoscopic gastritis, and histological gastritis. Symptomatic gastritis without organic disease has been proposed as an independent disease concept as functional dyspepsia, and gastritis = histological gastritis. In 2007, the Japan Gastroenterological Endoscopy Society established a study group to establish an endoscopic diagnosis of chronic gastritis with the aim of matching histological gastritis and endoscopic findings. Five articles were published in Dig Endosc, after which the endoscopic diagnosis of *H. pylori*-infected gastritis was established.[32–36] The Kyoto Classification of Gastritis was published to promote the endoscopic diagnosis of *H. pylori*-infected gastritis via endoscopic screening for gastric cancer.

In this study, atrophic gastritis, intestinal metaplasia, mucosal swelling, sticky mucus, hyperplastic polyps, and gastric cobblestone-like lesions were associated with positive gastric juice culture, while RAC and hematin were associated with negative gastric juice culture. Yoshi *et al.*[37] evaluated endoscopic findings according to the Kyoto classification of *H. pylori* infection status in 498 patients. They found that RAC [odds ratio (OR): 32.2], fundic gland polyps (OR: 7.7), and red streaks (OR: 4.7) in noninfected patients; map-like redness (OR: 12.9) in those with past infection; diffuse redness (OR: 26.8), mucosal swelling (OR: 13.3), and white mucus (OR: 10.2); and enlarged folds and tortuous folds (OR: 8.6) were significantly more likely to be associated with a diagnosis. Endoscopic findings of non-*H. pylori* bacteria-positive gastritis differed from endoscopic findings of *H. pylori*-infected gastritis in several respects.

Mucosal swelling and sticky mucus, as well as diffuse redness, are considered basic findings that indicate active gastritis (histologically, especially reflecting neutrophilic infiltration) and are reported as typical endoscopic findings of *H. pylori*-infected gastritis.[23] Although diffuse redness was not observed in this study, mucosal swelling and sticky mucus were observed in the oral bacteria-positive gastric mucosa, suggesting that inflammation may have been induced.

Hyperplastic polyps are thought to be inflammatory polyps formed by inflammatory cellular infiltration. Histologically, they are characterized by dilation of the glandular ducts and large and small cystic dilatations due to epithelial hyperplasia of the glandular fossa. Hyperplastic polyps were previously thought to be caused by inflammation associated with *H. pylori*-associated gastritis, but now, they should also be considered to be caused by inflammation associated with non-*H. pylori* bacterial gastritis.[38] It is necessary to examine whether PPI withdrawal and other measures can eliminate oral bacteria-associated hyperplastic polyps.

Hatta *et al.*[39] investigated differences between acid secretion and endoscopic findings in 223 subjects and reported that the acid secretion capacity was significantly greater in patients with endoscopic findings of hematin. RAC is an endoscopic image of regularly arranged collecting venules in the body of the stomach. RAC is considered to be an endoscopic finding that is not infected with oral bacteria, as reported as a typical endoscopic image of a normal stomach uninfected with *H. pylori*.[40]

In this study, atrophic gastritis and intestinal metaplasia may be related to the environment of low acid secretion capacity, and the intragastric environment is favorable for colony formation of indigenous oral or intestinal microorganisms due to long-term administration of PPIs. The pathogenesis of chronic gastritis may be related to the enrichment of oral or intestinal commensal microorganisms as the disease progresses from atrophic gastritis to intestinal metaplasia.[6,7]

Approximately 10.5% of gastric cobblestone-like lesions were reported in patients who had been taking PPIs for more than 6 months.[41] The frequency of detection of gastric cobblestone-like lesions was significantly greater in PPI-treated patients, and similar gastric cobblestone-like lesions were reported in P-CAB-treated patients.[42,43] The effect of PPI administration as well as non-*H. pylori* bacterial infection is likely to be significant.

Our results suggest that non-*H. pylori* bacteria may infect the stomach and cause gastric inflammation, especially in patients who use long-term gastric antisecretory drugs.

Limitations

This was a single-center retrospective study. This study is preliminarily based on a conventional gastric juice culture test, not on a genetic analysis, and does not reflect all gastric microbiota. This study used endoscopic diagnosis of gastritis based on the Kyoto classification of gastritis, and histological examination may be necessary in the future.

Author Contributions

Study concept and design, TK; methodology, TK and MS; software, RN, NN, MF, TK, and MS; investigation, TK, KY, YA, MH, and EI; formal analysis, TK and MS; drafting of the manuscript, TK and MS; critical revision of the manuscript, MS and TK; supervision, TI and TK; project administration, TK.

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Conflict of Interest

No potential conflicts of interest were disclosed.

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