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Comparison between Resectable *Helicobacter pylori*-Negative and -Positive Gastric Cancers

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See editorial on page 157.

Background/Aims: Controversy exists regarding the characteristics of *Helicobacter pylori* infection-negative gastric cancer (HPIN-GC). The aim of this study was to evaluate clinicopathologic features of HPIN-GC compared to *H. pylori* infection-positive gastric cancer (HPIP-GC) using a comprehensive analysis that included genetic and environmental factors. **Methods:** *H. pylori* infection status of 705 resectable gastric cancer patients was determined by the rapid urease test, testing for anti-*H. pylori* antibodies, histologic analysis and culture of gastric cancer tissue samples, and history of *H. pylori* eradication. HPIN-GC was defined as gastric cancer that was negative for *H. pylori* infection based on all five methods and that had no evidence of atrophy in histology or serology. **Results:** The prevalence of HPIN-GC was 4% (28/705). No significant differences with respect to age, sex, smoking, drinking, family history of gastric cancer or obesity were observed between the two groups. HPIN-GC tumors were marginally more likely to involve the cardia (14.3% for HPIN-GC vs 5.3% for HPIP-GC, p=0.068). The Lauren classification, histology, and TNM stage did not differ according to *H. pylori* infection status. Microsatellite instability was not different between the two groups, but p53 overexpression in HPIN-GC was marginally higher than in HPIP-GC (56.0% for HPIN-GC vs 37.0% for HPIP-GC, p=0.055). **Conclusions:** The prevalence of HPIN-GC was extremely low, and its clinicopathologic characteristics were similar to HPIP-GC. **(Gut Liver 2016;10:212-219)**

Key Words: *Helicobacter pylori*; Stomach neoplasms; Negative infection; Atrophy

INTRODUCTION

Helicobacter pylori is well recognized as a class I carcinogen that causes gastric cancer.[1] *H. pylori* infection induces superficial gastritis, which progresses to atrophic gastritis with loss of acid secretion and then to dysplasia and cancer.[2] A few prospective studies suggested that no gastric cancer developed in *H. pylori*-negative subjects.[3,4] However, several studies reported that no evidence of *H. pylori* infection was found in some patients with gastric cancer.[5-12]

The prevalence of *H. pylori* infection-negative gastric cancer (HPIN-GC) is considered very low, 13.8% in Italy[6] and 24.6% in Germany[5] and 0.66% to 10.6% in Korea[7,11,12] and Japan.[8-10] Previous studies have shown that HPIN-GC has known to have different clinicopathologic characteristics and prognosis compared to *H. pylori*-positive gastric cancer (HPIP-GC),[5-7] but those studies have not shown the consistent results because of the difference in the definition of *H. pylori* infection status. Determination of *H. pylori* infection status for gastric cancer is problematic because of false negatives for *H. pylori* tests and spontaneous disappearance in severely atrophic mucosa.[13,14] Some studies have focused on histologic and serologic atrophy in the definition of HPIN-GC in which patients with gastric cancer with severe gastric atrophy were considered as having a previous *H. pylori* infection.[5,7-9,11]

Our research group determined the *H. pylori* infection status using various methods (histology, rapid urease test, culturing, serology, and history of *H. pylori* eradication) and considered gastric atrophy assessing serum pepsinogen (PG) test and histologic evaluation of gastric atrophy and intestinal metaplasia to exclude the gastric cancer patients with possible past infection.[7,11] In our recent research, clinicopathologic features and

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molecular markers of gastric cancer were not significantly different according to *H. pylori* infection.[11]

In fact, gastric carcinogenesis is a complex and multifactorial process, in which infection with *H. pylori* plays a major role, resulting from the interplay between host genetic susceptibility factors and environmental factors. Several environmental factors including smoking,[15] salted and nitrated foods,[16] and heavy alcohol consumption[17] have known to independent strong risks for gastric cancer, and obesity has been recognized as a contributory factor in gastric cardia carcinogenesis.[18-20] Family history of gastric cancer[21] also has been found to be associated with the risk of developing gastric cancer. Besides, accumulations of genetic and epigenetic alterations that activate oncogenic and/or inactivate tumor-suppressor pathways also play crucial roles in the cellular tumorigenesis.

Therefore, more comprehensive study is needed to determine the characteristics of HPIN-GC, considering environmental factors, genetic susceptibility factors and molecular factors as well *H. pylori* infection. However, our previous study concluded the features and prognosis of HPIN-GC without assessing the important lifestyle factors such as smoking, drinking, obesity and family history of gastric cancer. To our knowledge, there is no extensive study on HPIN-GC assessing various factors, especially conducted in a large cohort.

From this background, we analyzed the clinicopathologic and molecular characteristics of HPIN-GC compared to those of HPIP-GC after added more patients to our existing cohort and surveyed the data about smoking, drinking, obesity and family history of gastric cancer.

MATERIALS AND METHODS

1. Patients

Between February 2006 and July 2014, 800 patients diagnosed as gastric cancer by endoscopic biopsy were prospectively enrolled at Seoul National University Bundang Hospital, South Korea. All patients were ethnically Koreans. Ninety-five patients who met the following criteria were excluded from this study: (1) patients who had not received endoscopic or surgical resection; (2) patients who had been lost from follow-up and had incomplete medical records; (3) patients who had not evaluated for serum PG. All patients provided informed consent, and this study protocol was approved by the Ethics Committee at Seoul National University Bundang Hospital (IRB number: B-1011/115-005).

2. Evaluation of gastric atrophy by serum PG test and histologic findings

In fasting serum collected from each patient, the concentrations of PG I and II were measured using a Latex-enhanced Turbidimetric Immunoassay (Shima Laboratories, Tokyo, Japan). Patients with PG I/II ratio ≤3.0 were regarded as the presence of atrophic gastritis.

Atrophic gastritis and intestinal metaplasia were determined by hematoxylin and eosin staining of the four biopsy specimens from each patient (one each from greater and lesser curvatures of antrum and body) for *H. pylori* evaluation. The histological features of the gastric mucosa were recorded using the updated Sydney scoring system (i.e., 0=none, 1=mild, 2=moderate, 3=severe). When the specimens were not prepared well enough to evaluate full-thickness gastric mucosa due to problems such as improper fixation, inaccurate orientation, and section inappropriateness, or whenever inflammation prevented a clear distinction between nonatrophic and atrophic phenotypes, samples were classified as "nonapplicable."

3. Determination of *H. pylori* infection status

Ten biopsy specimens were taken for three types of *H. pylori* testing (histology, rapid urease test, and culture) to determine the status of *H. pylori* infection.[22] Two biopsy specimens were taken from the greater curvature of both the antrum and body of the stomach, respectively, and three from both the lesser curvature of the antrum and body, respectively. Among the 10 specimens, two from the antrum and two from the body were fixed in formalin, and assessed for the presence of *H. pylori* by modified Giemsa staining. Another four specimens from the four gastric mucosa areas mentioned above were used for *H. pylori* culturing. The remaining two specimens from the lesser curvature of the antrum and body were used for the rapid urease test (CLO test; Delta West, Bentley, Australia). Current *H. pylori* infection was defined as a positive result from at least one of these three tests.[22]

To identify past *H. pylori* infection, the sero-positivity and eradication history were investigated. Sero-positivity was assessed by enzyme-linked immunosorbent assay (ELISA) for immunoglobulin G (IgG) specific for *H. pylori* antibody in each patient's serum (Genedia *H. pylori* ELISA; Green Cross Medical Science Corp., Eumsung, Korea). In addition, eradication history was evaluated in each patient by a questionnaire. If the patient has a history of *H. pylori* eradication or *H. pylori* serology was positive but no bacteria were found by histology, the rapid urease test, or culturing, the patient was diagnosed with a past *H. pylori* infection without current ongoing infection.

Because there was a high possibility of eliminating *H. pylori* infection owing to hostile mucosal condition,[23] cases with gastric atrophy were regarded as a past *H. pylori* infection. Therefore, among the patients with negative results from three biopsy-based tests, negative *H. pylori* serology and no history of *H. pylori* eradication, the true *H. pylori*-negative infection was diagnosed according as followed: PG I/II ratio >3 and (1) absent atrophy and intestinal metaplasia both in the antrum and body; (2) present mild atrophy either in the antrum or body but absent intestinal metaplasia both in the antrum and body; (3) nonapplicable for atrophy either in the antrum or body but absent

intestinal metaplasia both in the antrum and body; (4) absent atrophy both in the antrum and body, but present mild intestinal metaplasia either in the antrum or body. We determined the pathologic criteria of true *H. pylori*-negative infection base on the Operative Link on Gastritis Assessment (OLGA)[24] and Operative Link on Intestinal Metaplasia Assessment (OLGIM)[25] scoring system using the Updated Sydney Scoring System. Stage 0–1 OLGA and stage 0–1 OLGIM scores were classified as *H. pylori*-negative infection. If either or both samples in the antrum and corpus were nonapplicable for atrophy (four cases), having stage 0 OLGIM scores were classified as *H. pylori*-negative infection.

4. p53 immunohistochemistry

Sections 4-μm thick were cut from each tissue array block and then deparaffinized and dehydrated. Immunohistochemical staining was performed using an automatic immunostainer (BenchMark® XT; Ventana Medical Systems Inc., Tucson, AZ, USA) according to the manufacturer's instructions. The primary antibody used was mouse monoclonal antibody for p53 (DO7; Dako, Carpinteria, CA, USA). An antigen retrieval process was performed using microwave. Immunostaining 10% or more nuclear staining of tumor cells was considered positive.[26]

5. Microsatellite instability testing

Tumor DNA was extracted from paraffin-embedded tissues of tumors from individual patients. Normal DNA was extracted from the surrounding normal tissue. Five microsatellite markers (BAT-25, BAT-26, D2S123, D5S346, and D17S250), recommended by a National Cancer Institute workshop on microsatellite instability (MSI), were used to analyze paired normal and tumor DNA.[27] Polymerase chain reaction (PCR) was performed using a DNA auto-sequencer (ABI 3730 genetic analyzer; Applied Biosystems, Foster City, CA, USA). The shift of PCR products from tumor DNA was compared to that of DNA from normal mucosa. The size of each fluorescent PCR product was calculated using GeneMapper software (Applied Biosystems). According to the guideline of the National Cancer Institute, cases positive for ≥2 markers were considered as high-frequency MSI (MSI-H), while cases positive for <2 markers as low-frequency MSI (MSI-L) or microsatellite stable (MSS).[27,28]

6. Antiparietal cell antibody

Antiparietal cell antibody (APCA) was measured by indirect immunofluorescence on fixed tissue sections of mouse kidney stomach slide (Kallestad™; Bio-Rad Laboratories, Redmond, WA, USA).

7. Clinicopathologic characteristics

Baseline characteristics of subjects including age, gender, cigarette smoking, alcohol intake and familial history of gastric cancer in first-degree relatives were obtained by the questionnaire under the supervision of a well-trained interviewer. The subjects were classified into never, past and current smoker or drinker according to their smoking and alcohol histories about previous 6 months. The subjects' height and weight were checked or measured at the time of endoscopy in the endoscopy room, and the body mass index (BMI) was computed as weight in kilograms per square surface area in square meters (kg/m^2). According to the International Obesity Task Force criteria for the Asia-Pacific population, BMI scores were classified as follows: normal (<23), overweight (≥23 and <25), and obese (≥25 and <30).[29] The location, size, histologic features of the tumor, and TNM stage defined according to the seventh edition of AJCC Cancer Staging Manual were determined.[30] In patients underwent surgical resection, T and N classification were assessed based on the final pathological result and M classification was determined by surgical findings or computed tomography (CT) results. In patients underwent endoscopic resection, T classification was assessed by the final pathologic results, N and M classification were determined by CT findings. Early gastric cancer was defined as a tumor that was confined to the mucosa or submucosa regardless of lymph node (LN) involvement. Advanced gastric cancer was defined as a tumor that invaded the proper muscle or beyond.

8. Statistical analysis

To compare the clinicopathologic and molecular characteristics, Student t-test or chi-square test (Fisher exact test) was used for continuous variables and categorical variables, respectively. All analyses were carried out using the SPSS for Windows version 20.0 (SPSS Inc., Chicago, IL, USA). The results were considered statistically significant when p-values were less than 0.05.

RESULTS

1. Determination of the *H. pylori* infection status

A total of 705 patients with gastric cancer were enrolled during the study period. By the three biopsy-based tests, 445 patients were diagnosed with current *H. pylori* infection. One hundred sixty-four patients were diagnosed with past infection by anti-*H. pylori* IgG positivity or eradication history of *H. pylori*. In the 96 patients initially classified as HPIN-GC, we determined final 28 HPIN-GC patients according to our strict definition. Finally, of 705 patients with gastric cancer, 28 patients (4.0%) were categorized as HPIN-GC. The remaining 677 patients were categorized as HPIP-GC.

2. Clinicopathologic characteristics between *H. pylori*-infection positive and -negative gastric cancers

The clinical features between HPIP-GC group and HPIN-GC group were compared (Table 1). No statistical significant differences with regard to age, gender, familial history of gastric cancer in first-degree relatives, smoking status, alcohol status, and BMI categories were observed between two groups.

Table 1. Clinical Characteristics of the 705 Patients with Gastric Cancer according to the Final *Helicobacter pylori* Status

Clinical analysis, total	*H. pylori*-positive (n=677)	*H. pylori*-negative (n=28)	p-value
Male sex	452 (66.8)	18 (64.3)	0.785
Age, yr	59.3±12.0	56.0±13.6	0.161
Smoker			0.941
Never*	244 (36.1)	11 (39.3)	
Past	283 (41.9)	11 (39.3)	
Current	148 (21.9)	6 (21.4)	
Drinker*			0.688
Never	190 (28.2)	10 (35.7)	
Past	107 (15.9)	4 (14.3)	
Current	377 (55.9)	14 (50.0)	
BMI, kg/m^2			0.589
<23	312 (46.1)	14 (50.0)	
≥23 or <25	179 (26.4)	5 (17.9)	
≥25	186 (27.5)	9 (32.1)	
Family history of gastric cancer	139 (20.7)	5 (17.9)	0.717
Serum PG test			
PG I, ng/mL	67.2±50.7	66.6±35.9	0.949
PG II, ng/mL	25.6±28.8	11.8±5.5	<0.001†
PG I/II ratio	3.2±2.1	5.7±1.7	<0.001†
Treatment modalities			0.340
ESD	111 (16.4)	7 (25.0)	
Curative surgery	555 (82.0)	20 (71.4)	
Palliative surgery	11 (1.6)	1 (3.6)	

Data are presented as mean±SD or number (%).
BMI, body mass index; PG, pepsinogen; ESD, endoscopic submucosal dissection.
*Some data are missing, and missing values are not included; †Indicate statistical significance.

Table 2 showed the comparison of pathological characteristics between HPIP-GC group and HPIN-GC group. There were no statistical significant differences in tumor size, Lauren histotype and histologic type between HPIP-GC group and HPIN-GC group. We observed a higher frequency of cardia location in HPIN-GC group, but there was no significant difference (14.3% for HPIN-GC vs 5.3% for HPIP-GC, p=0.068). The depth of invasion was more advanced in HPIN-GC group (pT3-pT4, 32.1% for HPIN-GC vs 25.6% for HPIP-GC, p=0.611) without statistical significance. The proportion of lymph node metastasis in HPIN-GC group was more than that in HPIP-GC group, but there was no statistical significance (pN1-N3, 35.7% for HPIN-GC vs 31.2% for HPIP-GC, p=0.611). There was no significant difference in TNM stage although more advanced TNM stage was observed in HPIN-GC group compared with HPIP-GC group (stage II–IV, 39.9% for HPIN-GC vs 33.3% for HPIP-GC, p=0.973).

Table 2. Pathologic Characteristics of the 705 Patients with Gastric Cancer according to the Final *Helicobacter pylori* Status

Clinical analysis, total	*H. pylori*-positive (n=677)	*H. pylori*-negative (n=28)	p-value
Lauren histotype			0.522
Intestinal	380 (56.1)	14 (50.0)	
Diffuse or mixed	297 (43.9)	14 (50.0)	
Histologic type			0.937
Tubular ADC, W/D	130 (19.2)	5 (17.9)	
Tubular ADC, M/D	229 (33.8)	9 (32.1)	
Tubular ADC, P/D	180 (26.6)	9 (32.1)	
Signet ring cell carcinoma	116 (17.1)	5 (17.9)	
Mucinous adenocarcinoma	13 (1.9)	0	
Others*	9 (1.3)	0	
Tumor location			0.068
Cardia†	36 (5.3)	4 (14.3)	
Noncardia	641 (94.7)	24 (85.7)	
Tumor size, cm	3.7±3.0	3.3±2.1	0.633
Depth of invasion			0.435
pT1–T2	504 (74.4)	19 (67.9)	
pT3–T4	173 (25.6)	9 (32.1)	
Lymph node metastases			0.611
pN0	466 (68.8)	18 (64.3)	
pN1–N3	211 (31.2)	10 (35.7)	
Distant metastases			1.000
Absent	650 (96.0)	27 (96.4)	
Present	27 (4.0)	1 (3.6)	
TNM stage			0.539
I	449 (66.3)	17 (60.7)	
II, III, IV	228 (33.7)	11 (39.3)	

Data are presented as mean±SD or number (%).
ADC, adenocarcinoma; W/D, well differentiated; M/D, moderately differentiated; P/D, poorly differentiated.
*Includes papillary adenocarcinoma, adenosquamous carcinoma, and undifferentiated carcinoma; †Located within 2 cm below the gastroesophageal junction.

3. Molecular characteristics between *H. pylori*-infection positive and -negative gastric cancers

We examined p53 immunohistochemistry and PCR-based MSI testing in gastric cancer tissues. Some patients were excluded from this analysis because of incomplete of record or insufficient remaining tissue. MSI-H was more frequently observed in HPIN-GC group, but it was not significantly different (16.7% for HPIN-GC vs 8.3% for HPIP-GC, p=0.195) (Table 3). Positivity of p53 expression was more frequent in HPIN-GC group than HPIP-GC group without statistical significance (56.0% for HPIN-GC vs 37.0% for HPIP-GC, p=0.055) (Table 3).

Table 3. Molecular Characteristics of the Gastric Cancer Patients according to the Final *Helicobacter pylori* Status

Characteristic	*H. pylori*-positive*	*H. pylori*-negative*	p-value
MSI			0.195
MSI-high	41 (8.3)	3 (16.7)	
MSI-low/MSS	453 (91.7)	15 (83.3)	
Total	494 (100.0)	18 (100.0)	
p53			0.055
Positive	240 (37.0)	14 (56.0)	
Negative	408 (63.0)	11 (44.0)	
Total	648 (100.0)	25 (100.0)	

Data are presented as number (%).
MSI, microsatellite instability; MSS, microsatellite stable.
*Some data were missing, and missing values were not included.

4. APCA in *H. pylori*-positive gastric cardia cancer

APCAs were measured in the 30 patients with *H. pylori*-positive gastric cardia cancers to exclude the autoimmune atrophic gastritis. No one was positive for APCA.

DISCUSSION

There have been a few studies on the clinicopathologic features and prognosis of gastric cancer according to *H. pylori* infection status.[5-12] The prevalence of HPIN-GC varied among studies according to definition of *H. pylori*-negative infection. In fact, there are many methods provided for detecting *H. pylori*, including histologic examination, rapid urease test, culture, anti-*H. pylori* antibody. However, diagnostic tests have been challenged because *H. pylori* bacteria are spontaneously disappeared in severely atrophic mucosa and advanced gastric diseases once they have caused gastric atrophy and intestinal metaplasia after long-time colonization,[23] and the spontaneous seroconversion of anti-*H. pylori* antibodies often occurs.[13] Therefore, it is difficult to determine whether patients with gastric cancer who had advanced gastric atrophy and intestinal metaplasia have had previous *H. pylori* infection based on conventional *H. pylori* test. To overcome this problem, the absence of serologic atrophy (PG I ≤70 ng/mL and/or PG I/II ratio ≤3.0) and histologic atrophy was included to define HPIN-GC in Korean and Japanese studies and the prevalence of HPIN-GC in these studies were found to less than 10%.[7,9,11] Moreover, in recent two studies conducted in Japan, cases with even active gastritis with neutrophil infiltration but without *H. pylori* detection in the histologic examination[8] or cases with endoscopic atrophy without serologic and histologic atrophy[10] were considered as a previous *H. pylori* infection, the prevalence of HPIN-GC was extremely low (0.42% and 0.66%).

In the present study, the presence of moderate/severe atrophy and intestinal metaplasia in histology and serologic atrophy (PG I/II ≤3.0) were regarded as possible past *H. pylori* infection to detect past infection with *H. pylori* more precisely. Therefore, the actual prevalence of HPIN-GC in present study was calculated as 4.0% (28/705) which was comparable to those reported in previous Korean and Japanese studies.

Recently, Kwak *et al*.[12] reported the prevalence of HPIN-GC was 2.3% using histology, rapid urease test, anti-*H. pylori* antibody and histologic examination of gastric atrophy and intestinal metaplasia, but serum PG level and culturing were not included.[12] Also they compared the characteristics of HPIN-GC each to those of gastric cancer with current *H. pylori* infection and past *H. pylori* infection, and did not show the data about the family history of gastric cancer and lifestyle factors such as smoking, drinking, and obesity.

In the present study, HPIN-GC did not have different characteristics with regard to well-known independent risk factors of gastric cancer such as age, gender, and family history of gastric cancer. Smoking and alcohol status were not significantly different between two groups, and obesity was not significantly more common in HPIN-GC. No previous studies comparing the features of HPIN-GC and those of HPIP-GC have shown the data about obesity, smoking, drinking, and family history of gastric cancer yet. But we did not have data available regarding salty and nitrated food intake, further researches including dietary habit are needed to establish the impact of lifestyle factors on HPIN-GC.

In the literature, the advanced T and N stages,[6,7,11,31] and histologically diffuse type[8,12] were frequently reported in HPIN-GC but the results were not consistent among the studies. In addition, the negative *H. pylori* infection was reported as an independent poor prognostic factor for survival in gastric cancer in two Western studies, but these studies did not consider gastric atrophy and intestinal metaplasia.[5,6] In our earlier research[11] and present research in which histologic and serologic gastric atrophy were considered, the advanced T and N stages were found in HPIN-GC, but there was no significant difference. Although we could not collect patient survival data in the present study, considering the TNM stage of their disease, poor prognosis was not expected in HPIN-GC. And the pathologic features of HPIN-GC such as histology and Lauren's classification were not different from those of HPIN-PC. A higher frequency of cardia location was observed in HPIN-GC although there is no significant difference (14.3% for HPIN-GC vs 5.3% for HPIP-GC, p=0.068) in present study. Some previous studies reported that the HPIN-GC arose more often upper location.[5,6,32] There is a possibility that the no statistical significances might be originated from the small number of HPIN-GC, therefore a cardia location in HPIN-GC would be significantly frequent with large enough numbers of HPIN-GC patients.

The genetic and epigenetic changes in oncogenes and tumor suppressor genes, cell cycle regulators, and DNA repair genes have known to contribute to gastric carcinogenesis.[33] Recent

molecular biological studies have revealed that there are at least two distinct genetic pathways for gastrointestinal carcinogenesis, the suppressor and mutator pathways.[34] Mutator pathway, characterized by MSI, is suggested to play an important role in the tumorigenesis of the foveolar type, whereas the suppressor pathway, represented by p53 alteration, could participate in the tumorigenesis of complete-type intestinal metaplastic phenotype gastric adenocarcinomas.[34] MSI is defined as length changes of microsatellites, which are repeating sequences of 1–6 base pairs of DNA and caused by an impairment of DNA mismatch repair system.[33] The frequency of MSI in gastric cancer has been reported from 9.5% to 44% and some studies have shown associations between MSI-H in gastric cancer and intestinal type, older age, distal location, lower prevalence of LN metastasis, and better prognosis.[33] Though the number of cases with HPIN-GC was limited in the present study, the proportion of MSI-H was not significantly different between HPIN-GC and HPIP-GC.

The alteration or inactivation of p53 tumor suppressor gene, which is the most frequently mutated gene in human cancers, allows a cell with damaged DNA to escape from normal growth, resulting in cancer development.[33,35] The positivity rate of p53 expression in gastric cancer has been reported to range from 4% to 71%, and the association with intestinal-type gastric cancer,[26,36,37] higher frequency of cardia cancer[38,39] and poor prognosis[40] has also been reported. Recent *in vivo* studies have demonstrated that *H. pylori* can affect p53 function through mutation-independent mechanism as well as mutational mechanism,[41-44] especially common in patients infected with cytotoxin-associated gene A positive strain of *H. pylori*. Because of prolonged half-life of the mutant p53 protein compared with the wild-type p53, immunochemical overexpression of p53 protein in tumors has been interpreted as a surrogate for p53 mutation. There have been a few studies that showed a higher immunochemical staining of p53 expression in HPIP-GC than in HPIN-GC.[45-47] In current study, genetic studies for the confirmed detection of p53 mutations were not conducted, but no statistical significant difference in expression of p53 were observed between HPIN-GC and HPIP-GC (56.0% for HPIN-GC vs 37.0% for HPIP-GC, p=0.055). The higher frequency of p53 overexpression in HPIN-GC might be due to the features of cardia cancer because cardia cancer among HPIN-GC in this study was relatively high (14.3%). If the number of HPIN-GC is increased then there might be a possibility of difference. Thus further research is necessary.

We considered atrophy and intestinal metaplasia as a result of long-standing *H. pylori* infection. However, autoimmune gastritis also causes atrophic gastritis.[48] Autoimmune atrophic gastritis is characterized by a chronic atrophic gastritis limited to the mucosa of the corpus and fundus and by a marked diffuse atrophy of parietal and chief cells. It is associated with serum antiparietal and anti-intrinsic factor antibodies and the cause of pernicious anemia. In current study, APCAs were measured in the 30 patients with *H. pylori*-positive gastric cardia cancers, but no one was positive in APCA. Three of 36 patients with *H. pylori*-positive gastric cardia cancers had negative *H. pylori* tests but had gastric atrophy. Although APCA was measured in only one patient, we could exclude autoimmune gastritis in these three patients because chronic atrophic gastritis was present in the antrum.

Gastric cancer could develop in the hereditary diffuse gastric cancer, irrespective of *H. pylori* infection. Hereditary diffuse gastric cancer is extreme rare in Korea[49] and we did not find any suspected hereditary diffuse gastric cancer patient. That is, we could not find any gastric cancer patient below 50 years old who has at least three gastric cancer family members over grandfather, father and son or daughter.

Our study has several limitations. Although we used the combination of multiple diagnostic methods to minimize the possibility of false-negative *H. pylori* testing results, a PCR-based assay was not available. PCR may have been more sensitive compared with the other diagnostic techniques but is not usually used in the clinical setting. Only gastric cancers treated by endoscopic and surgical resection were included in this study. The advanced-stage gastric cancers that underwent palliative chemotherapy or conservative treatment might be excluded selectively.

In this study, we showed that gastric cancers that are not associated with *H. pylori* infection are rare in Korea and according to *H. pylori* infection status, gastric cancers contain similar clinicopathologic characteristics, lifestyle factors, and molecular features such as MSI and p53. This finding suggests that biological and genetic factors except for MSI and p53 might be involved in gastric cancer regardless of *H. pylori* infection. Therefore, to understand the exact carcinogenesis of HPIN-GC and to identify early diagnostic markers of HPIN-GC, further studies on molecular cancer genetics are crucial.

CONFLICTS OF INTEREST

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

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