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Postoperative *Helicobacter pylori* Infection as a Prognostic Factor for Gastric Cancer Patients after Curative Resection

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Background/Aims: Few studies have evaluated the effect of *Helicobacter pylori* infection on the prognosis of patients diagnosed with gastric cancer (GC) after curative surgery. We investigated the association between the *H. pylori* infection status and clinical outcome after surgery. **Methods:** We assessed the *H. pylori* status of 314 patients who underwent curative resection for GC. The *H. pylori* status was examined using a rapid urease test 2 months after resection. Patients were followed for 10 years after surgery. **Results:** An *H. pylori* infection was observed in 128 of 314 patients. The median follow-up period was 93.5 months. A Kaplan-Meier analysis indicated that patients with *H. pylori* had a higher cumulative survival rate than those who were negative for *H. pylori*. Patients with stage II cancer who tested negative for *H. pylori* were associated with a poor outcome. In a multivariate analysis, *H. pylori*-negative status was a significant independent prognostic factor for poor overall survival. **Conclusions:** Having a negative *H. pylori* infection status seems to indicate poor prognosis for patients with GC who have undergone curative resection. Further prospective controlled studies are needed to evaluate the mechanism by which *H. pylori* affects GC patients after curative surgery in Korea. **(Gut Liver 2017;11:635-641)**

Key Words: *Helicobacter pylori*; Stomach neoplasms; General surgery; Prognosis

INTRODUCTION

In Korea, gastric cancer (GC) is the second most common cancer.[1] Because GC is not a homogenous disease, the prognosis of patients with GC is diverse and is currently based on histology and tumor stage. *Helicobacter pylori* infection is closely associated with GC carcinogenesis. The International Agency for Research on Cancer, a subdivision of the World Health Organization (WHO), recognizes *H. pylori* as a group I carcinogen for gastric carcinoma.[2] However, there are also GCs which are not related to *H. pylori* infection. *H. pylori*-negative GCs comprise 2% to 10% of all GCs.[3] Previous studies suggest that a negative *H. pylori* status is correlated with more advanced disease than a positive *H. pylori* status.[4,5] In addition, there are some reports that a negative *H. pylori* status is a prognostic factor of poor outcome in patients with GC after gastrectomy.[6-8] Meimarakis *et al*.[6] reported that relapse-free and overall survival of patients with positive *H. pylori* status are significantly higher than in patients with negative *H. pylori* status after curative resection. They evaluated the *H. pylori* status of 166 patients after curative resection to treat GCs. Among them, 41 patients (24.7%) were negative for *H. pylori*. And, they suggest that tumor-specific immune responses might be downregulated in patients who are negative for *H. pylori*.[6] In Korea, there was a report that a negative *H. pylori* status is the most significant independent factor to predict poor prognosis in patients with locally advanced GC treated with adjuvant chemotherapy after curative surgery.[8] Kang *et al*.[8] investigated the *H. pylori* infection status in 274 locally advanced GC patients. Of these, 108 patients (39.4%) were negative for *H. pylori*. In this study, we aimed to investigate the *H. pylori* infection status and evaluate the clinical significance of *H. pylori* infection for patients with GC after they received curative surgery.

MATERIALS AND METHODS

1. Patients

Between February 1996 and December 2012, 314 patients were tested for *H. pylori* infection using a rapid urease test at

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least 2 months after curative resection to treat GC at Severance Hospital. Curative resection was defined according to the Japanese GC treatment guidelines.[9] Patients were regarded as positive for *H. pylori* if they were positive by the rapid urease test. Patients were evaluated in terms of age, sex, history of chemotherapy or radiotherapy, and survival. The investigated variables also included tumor location, tumor size, depth of tumor invasion, lymph node metastasis, histological classification, lymphovascular invasion, and perineural invasion. The following patients were excluded: (1) patients that received a total gastrectomy; (2) patients who were not given the rapid urease test after gastrectomy; (3) patients with distant metastasis (M1); (4) patients that received chemotherapy prior to gastrectomy; and (5) patients who received *H. pylori* eradication therapy before curative resection. The pathologic stage of the tumor was determined according to the standards set by the seventh American Joint Committee on Cancer (AJCC).[10] The Institutional Review Board of Severance Hospital approved this study.

2. Follow-up

During the first 2 years after gastrectomy, patients were assessed every 3 months by clinical examination, routine blood tests, and tumor markers; abdominal computed tomography and endoscopy were performed every 6 months. During the next 3 years, patients were examined every 6 months and received endoscopy every 12 months.

3. Statistical analysis

The chi-square and Fisher exact tests were used to compare the clinicopathological factors between groups based on their *H. pylori* status. The t-test was used for noncategorical variables in the intergroup comparisons of the clinicopathological characteristics. Results were considered significant if $p<0.05$. For the multivariate analysis, variables with $p<0.05$ on the univariate analysis and clinically important variables including age, gender, tumor size, and tumor stage were entered. The Kaplan-Meier method was used to determine the overall survival rates and the log-rank test was used to analyze differences in the survival curve. A Cox proportional hazards model and multivariate analyses were used to determine the risk assessment. All statistical analyses were performed using SPSS version 12.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

1. Association among clinicopathological characteristics and *H. pylori* infection

Two hundred and ten men and 104 females were enrolled in this study. Their mean age was 55.4 years (standard deviation, ±11.5). There were no differences in age, gender, tumor size, tumor location, T stage, N stage, AJCC stage, WHO classification, Lauren classification, lymphovascular invasion, and perineural invasion between the *H. pylori*-positive and -negative groups (Table 1).

Table 1. Association between Clinicopathologic Findings and *Helicobacter pylori* Status

Variable	*H. pylori*		p-value
	Positive (n=128)	Negative (n=186)	
Age, yr	54.4±10.8	56.2±12.0	0.174
Sex			0.807
Male	87 (68.0)	123 (66.1)	
Female	41 (32.0)	63 (33.9)	
Tumor size, mm	29.2±19.9	27.6±19.3	0.488
Tumor location			1.000
Middle third	48 (37.5)	71 (38.2)	
Lower third	80 (62.5)	115 (61.8)	
T stage			0.490
T1	79 (61.7)	130 (69.9)	
T2	19 (14.8)	20 (10.8)	
T3	16 (12.5)	20 (10.8)	
T4	14 (10.9)	16 (8.6)	
N stage			0.526
N0	91 (71.1)	143 (76.9)	
N1	21 (16.4)	20 (10.8)	
N2	10 (7.8)	15 (8.1)	
N3	6 (4.7)	8 (4.3)	
AJCC stage			0.346
I	86 (67.2)	136 (73.1)	
II	29 (22.7)	30 (16.1)	
III	13 (10.2)	20 (10.8)	
WHO classification			0.992
Well differentiated	23 (18.0)	35 (18.8)	
Moderately differentiated	42 (32.8)	64 (34.4)	
Poorly differentiated	34 (26.6)	46 (24.7)	
Signet ring cell	26 (20.3)	36 (19.4)	
Mucinous	3 (2.3)	5 (2.7)	
Lauren classification			0.321
Intestinal	63 (49.2)	102 (54.8)	
Diffuse	62 (48.4)	76 (40.9)	
Mixed	3 (2.3)	8 (4.3)	
Lymphovascular invasion			0.268
Absent	112 (87.5)	153 (82.3)	
Present	16 (12.5)	33 (17.7)	
Perineural invasion			0.842
Absent	116 (90.6)	170 (91.4)	
Present	12 (9.4)	16 (8.6)	

Data are presented as mean±SD or number (%).
AJCC, American Joint Committee on Cancer; WHO, World Health Organization.

2. Association between overall survival and *H. pylori* infection status

The median duration for follow-up assessments was 93.5 months (range, 18 to 208 months). The 10-year overall survival rate was 95.3%, 96.5%, and 69.8% for stage I, II, and III cancers, respectively. In the univariate analysis, the 10-year overall survival rate was 95.1% and 91.5% for patients positive and negative for *H. pylori*, respectively (p=0.030) (Fig. 1). When examining the different stages of cancer, the overall survival rate for patients negative for *H. pylori* was lower than those who tested positive for *H. pylori* (Fig. 2). In the univariate analysis, old age and negative for *H. pylori* infection status were correlated with lower overall survival rate (Table 2). We performed subgroup analyses based on the different clinicopathological factors. Old age, lower third location, advanced T stage (T3/4), and negative for *H. pylori* infection status were correlated with poor overall survival rates (Table 3). In the multivariate analysis, old age (hazard ratio, 3.02; 95% confidence interval, 1.22 to 7.47; p=0.017) and negative *H. pylori* status (hazard ratio, 2.95; 95% confidence interval, 1.14 to 7.66, p=0.026) were independent prognostic factors of poor overall survival (Table 4).

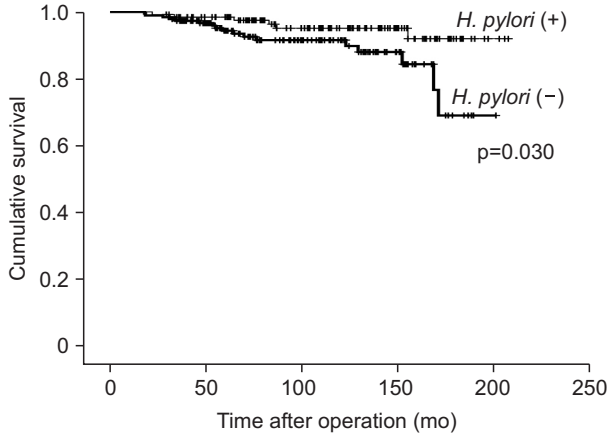

Fig. 1. Effect of *Helicobacter pylori* status on overall survival of gastric cancer after gastrectomy.

3. Association between disease-specific survival and *H. pylori* status

We analyzed the disease-specific survival rates between *H. pylori*-negative and -positive GC patients. The 10-year disease-specific survival rates for patients with negative and positive *H. pylori* status were 93.9% and 95.5%, respectively (Fig. 3). In ad-

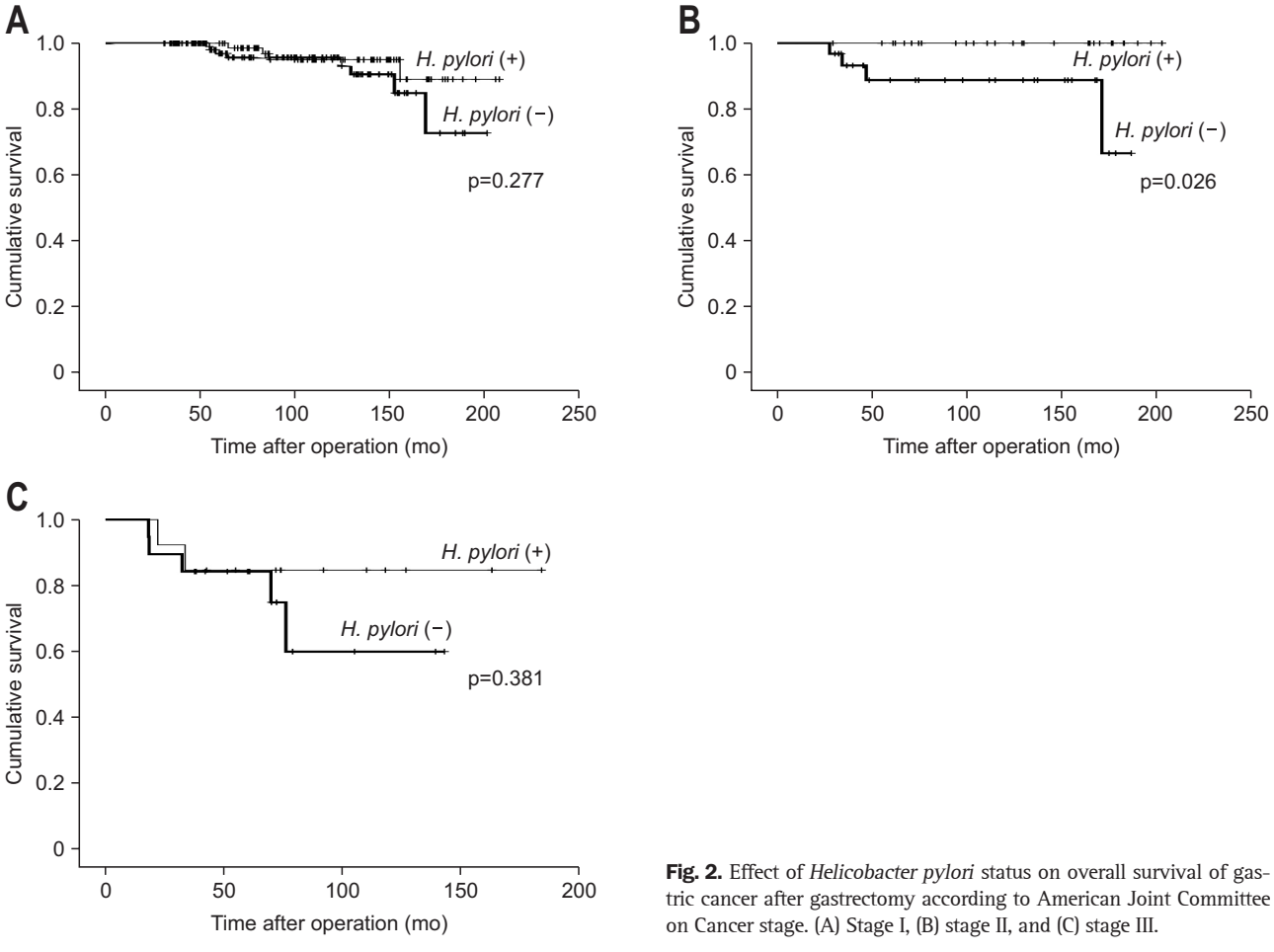

Fig. 2. Effect of *Helicobacter pylori* status on overall survival of gastric cancer after gastrectomy according to American Joint Committee on Cancer stage. (A) Stage I, (B) stage II, and (C) stage III.

Table 2. Univariate Analysis of Overall Survival

Factor	No. of patients	10-Year OS rate, %	HR (95% CI)	p-value
Age, yr				0.011
≤56	166	96.3	Reference	
>56	148	89.2	3.21 (1.31–7.86)	
Sex				0.736
Male	210	92.8	1.17 (0.48–2.83)	
Female	104	93.8	Reference	
Tumor size, mm				0.081
<20	110	98.4	Reference	
≥20	204	90.3	2.62 (0.89–7.69)	
Tumor location				0.068
Middle third	119	87.7	Reference	
Lower third	195	96.7	2.16 (0.94–4.92)	
WHO classification				0.882
Well differentiated	58	93.5	Reference	
Moderately differentiated	106	93.0	0.78 (0.25–2.47)	
Poorly differentiated	80	91.6	1.04 (0.33–3.28)	
Signet ring cell	8	96.1	0.63 (0.15–2.63)	
Mucinous	62	87.5	1.77 (0.21–15.22)	
H. pylori status				0.036
Positive	186	95.1	Reference	
Negative	128	91.5	2.73 (1.07–6.98)	
T stage				0.132
T1/2	209	95.2	Reference	
T3/4	105	88.7	1.88 (0.83–4.28)	
N stage				0.315
N0	234	94.4	Reference	
N1–3	80	89.1	1.56 (0.66–3.71)	

OS, overall survival; HR, hazard ratio; CI, confidence interval; WHO, World Health Organization; *H. pylori*, *Helicobacter pylori*.

dition, we evaluated the disease-specific survival rates according to the T and N stages. For patients with T2–4 stages of disease, the disease-specific survival rate for patients with a negative *H. pylori* status was significantly lower than in those with a positive *H. pylori* status (Fig. 4).

DISCUSSION

According to Correa's hypothesis, *H. pylori* infection is closely associated with the development of GC.[11] However, there are few reports about the role of *H. pylori* infection in GC patients after curative surgical resection. Currently, there are no guidelines regarding the treatment of *H. pylori* infection after gastrectomy and the effects of treatment are unknown. To our knowledge, Lee *et al*.[4] first reported that *H. pylori* seropositive GC patients showed a better prognosis after gastrectomy. There are two prospective studies that investigated the prognostic role of *H. pylori* infection.[6,7] In Germany, Meimarakis *et al*.[6] showed that a negative *H. pylori* status is an independent prognostic factor of poor relapse-free and overall survival in patients with GC after curative surgery. In patients with early-stage GC, the overall survival of patients who were positive for *H. pylori* was significantly higher than those who were negative.[6] They evaluated *H. pylori* infection by bacterial culture, serologies, and histologies. Previous study reported that patients with a negative for *H. pylori* infection status have a worse prognosis after curative surgery. *H. pylori* infection was determined by polymerase chain reaction analysis for the *vacA* gene and a serology of *H. pylori* and CagA antibodies. *H. pylori* infection is correlated with long-term survival of patients with early as well as advanced pT disease.[7] In this study, the 10-year overall survival of *H. pylori*-negative GC patients after curative resection was significantly lower than *H. pylori*-positive GC patients, which is similar to data observed in the previous studies. The prognostic impact of a negative for *H. pylori* infection status was significant for some of the subgroups such as old age, lower third location, and advanced T

Table 3. Comparison of the Overall Survival and Hazard Ratios for Overall Survival Rate between *Helicobacter pylori*-Negative and *H. pylori*-Positive Gastric Cancer Patients according to Clinicopathologic Factors

Factor	10-Year OS rate, %		HR (95% CI)	p-value
	H. pylori-positive	*H. pylori*-negative		
Age, yr				
≤56	96.6	96.1	1.33 (0.29–6.03)	0.710
>56	93.1	86.4	3.88 (1.09–13.79)	0.036
Sex				
Male	94.1	91.6	2.28 (0.78–6.71)	0.134
Female	97.0	91.6	4.73 (0.57–39.30)	0.151
Tumor size, mm				
<20	96.7	100.0	3.21 (0.33–31.48)	0.317
≥20	94.4	86.8	2.68 (0.96–7.48)	0.060
Tumor location				
Middle third	89.7	86.4	1.47 (0.48–4.53)	0.504
Lower third	98.8	95.0	9.60 (1.19–77.50)	0.034
WHO classification				
Well differentiated	95.2	92.1	3.29 (0.37–29.52)	0.288
Moderately differentiated	92.4	93.3	2.28 (0.44–11.85)	0.326
Poorly differentiated	94.0	89.1	2.23 (0.43–11.64)	0.340
Signet ring cell	100.0	93.0	2.80 (0.22–35.81)	0.428
Mucinous	100.0	80.0	42.98 (0.00–)	0.661
T stage				
T1/2	94.9	95.6	1.90 (0.57–6.36)	0.300
T3/4	95.8	81.3	4.77 (1.02–22.28)	0.047
N stage				
N0	95.5	93.8	2.52 (0.80–8.00)	0.116
N1–3	94.6	82.4	3.35 (0.67–16.84)	0.142

OS, overall survival; HR, hazard ratio; CI, confidence interval; WHO, World Health Organization.

Table 4. Multivariate Analysis of Overall Survival

Factor	HR (95% CI)	p-value
Age, yr		0.017*
≤56	Reference	
>56	3.02 (1.22–7.47)	
Sex		0.835†
Male	1.10 (0.44–2.74)	
Female	Reference	
Tumor size, mm		0.236†
<20	Reference	
≥20	2.03 (0.63–6.57)	
H. pylori status		0.026*
Positive	Reference	
Negative	2.95 (1.14–7.66)	
T stage		0.319†
T1	Reference	
T2–4	1.68 (0.60–4.70)	
N stage		0.926†
N0	Reference	
N1–3	1.05 (0.37–2.96)	

HR, hazard ratio; CI, confidence interval; *H. pylori*, *Helicobacter pylori*.
*Variables with p<0.05; †Clinically important variables.

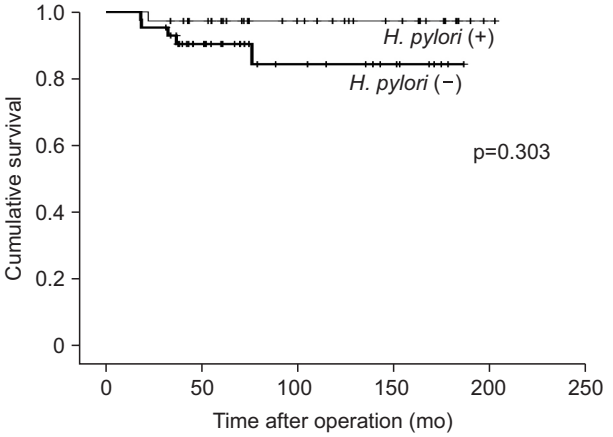

Fig. 3. Effect of *Helicobacter pylori* status on disease specific survival of gastric cancer after gastrectomy.

stage. In the multivariate analysis, old age and negative for *H. pylori* infection status were independent prognostic factors for poor overall survival of GC patients after curative surgery. We assessed *H. pylori* status using a rapid urease test 2 months after the curative surgery for GC.

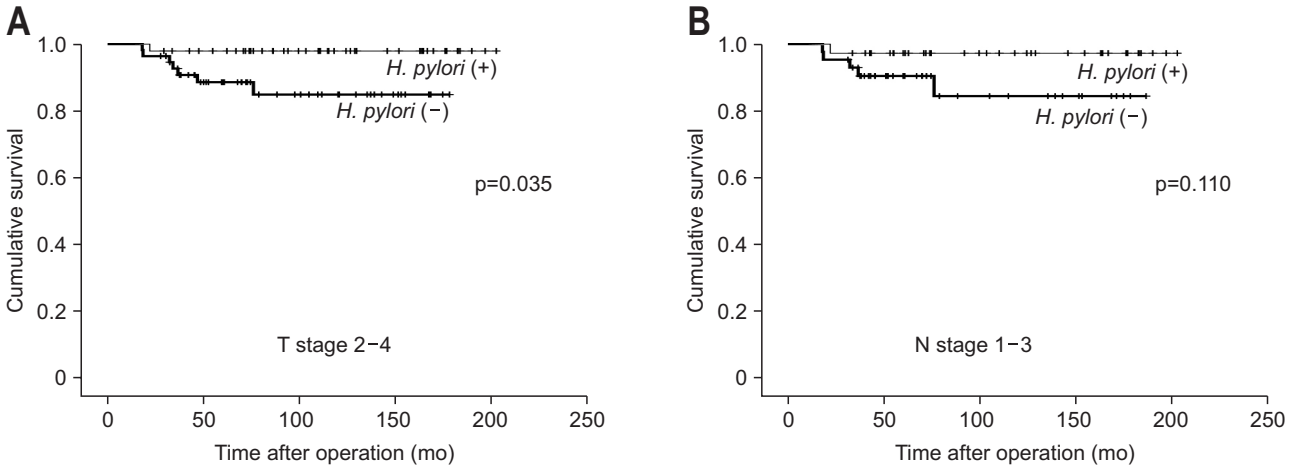

Fig. 4. Effect of *Helicobacter pylori* status on disease specific survival of gastric cancer after gastrectomy according to T and N stage. (A) T stage 2–4 and (B) N stage 1–3.

The reason that negative for *H. pylori* infection status is correlated with poor prognosis in GC patients after curative surgery is not well known. There are several possible explanations. Immune responses caused by *H. pylori* could evoke antitumor immunity. A previous report shows that patients negative *H. pylori* status have more numbers of cells expressing OX40 in cancerous tissue than those positive *H. pylori* status. OX40 directly modulates immune suppression mediated by regulatory T cells.[12] Thus, the authors contend that tumor-specific immune responses are downregulated in patients without *H. pylori* infection, in consequence of increased infiltration of OX40 positive cells.[6] $CD4^+$ and $CD8^+$ T cells are increased in the presence of *H. pylori*, suggesting that tumor antigens might induce stronger immune reactions during *H. pylori* infection.[13] Further, Xue *et al*.[14] assume that *H. pylori* components either mimic or bind to specific receptors or surface molecules on gastric epithelial cells, which could result in autoantibodies. These autoantibodies could recognize GC cells which may display the mimic *H. pylori* antigens.[14]

A second plausible reason is the part of microsatellite instability (MSI) in *H. pylori*-positive GC. A previous study showed that patients with MSI are more likely to have active *H. pylori* infection than those with stable tumors.[15] Further, previous study reported that alterations in MSI is associated with a higher rate of *H. pylori* infection, a better postoperative survival, and less lymph node metastasis.[16] However, some authors argue whether *H. pylori* infection status has prognostic value or not. They suggest that negative for *H. pylori* infection status might be correlated with more advanced tumor status.[5,17] In this study, there were no differences in the clinicopathological characteristics between the *H. pylori*-positive and -negative groups.

Our study has some limitations. First, this is a retrospective study and *H. pylori* infection was evaluated using a rapid urease test of the remnant stomach during the endoscopic examinations. Therefore, patients who underwent total gastrectomy because of proximally located GC were excluded from this study. And, we could not evaluate *H. pylori* infection by histology, urea breath test, and serology. Second, the 10-year overall survival of patients was 93.1%, which is quite high. This is mainly due to the high proportion of patients with stage I cancer (70.7%). To our knowledge, our study was the largest reported that analyzed the association between *H. pylori* infection and prognosis of GC patients who underwent curative surgery with long-term follow-up. In this study, patients negative for *H. pylori* infection were significantly associated with poor prognosis.

In conclusion, negative for *H. pylori* infection status appeared to be an indicator of poor prognosis in GC patients treated with curative surgery. Further prospective studies that include patients with advanced stages of cancer are needed to examine the effect of *H. pylori* status on the prognosis of GC patients after curative surgery.

CONFLICTS OF INTEREST

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

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