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Original Article

Clinical Significance of Epstein-Barr Virus and *Helicobacter pylori* Infection in Gastric Carcinoma

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Background/Aims: Epstein-Barr virus (EBV) and *Helicobacter pylori* (HP) coinfection may synergistically induce severe inflammatory responses in the stomach tissue, increasing the risk of developing gastric cancer. We aimed to analyze the effect of EBV and HP coinfection on the clinicopathologic features and prognosis of gastric cancer, as well as to evaluate the role of EBV infection in non-gastric carcinoma with lymphoid stroma (non-GCLS).

Methods: Overall, 956 patients who underwent surgery for gastric cancer between September 2014 and August 2015 were eligible and divided into groups, according to GCLS morphology, EBV infection, and HP infection. Clinicopathologic characteristics and oncologic outcomes were analyzed retrospectively.

Results: EBV and HP coinfection was significantly associated with male sex, proximal location, GCLS morphology, and equivocal p53 expression (p<0.001). Multivariate analysis revealed that EBV infection alone (hazard ratio [HR], 0.362; 95% CI, 0.131 to 0.996; p=0.049) and lower third location (HR, 0.624; 95% CI, 0.413 to 0.943; p=0.025) were inversely correlated with overall survival. During median follow-up period of 72 months, overall survival rate was not significantly different between the EBV and HP coinfection group and others (97.6% vs 86.8%, log-rank p=0.144). In non-GCLS patients (n=920), overall survival rate was not significantly different between the EBV infection group and others (96.9% vs 86.4%, log-rank p=0.126).

Conclusions: EBV and HP coinfection is not an independent prognostic factor for gastric cancer. EBV infection status, regardless of HP infection, affects the clinicopathologic features of all types of gastric cancer. However, it does not lead to a significant difference in overall survival of non-GCLS patients. **(Gut Liver 2023;17:69-77)**

Key Words: Coinfection; Gastric cancer; Gastric carcinoma with lymphoid stroma; Prognosis

INTRODUCTION

According to Global Cancer Statistics 2020, gastric cancer is the fifth most commonly diagnosed cancer and the fourth leading cause of cancer mortality worldwide. Genetic and environmental risk factors play important roles in gastric carcinogenesis.[1] Epstein-Barr virus (EBV), a human herpes virus with oncogenic activity, is reported to be a significant risk factor for gastric cancer subtypes in the Cancer Genome Atlas network.[2] It is particularly associated with gastric carcinoma with lymphoid stroma (GCLS), which is characterized by undifferentiated carcinoma with prominent lymphoid infiltration.[3] According to a previous study, EBV+ GCLS showed a better prognosis, while EBV– GCLS was similar to conventional gastric cancer (non-GCLS).[4] However, since EBV infection in non-GCLS is rare, few studies discuss the effect of EBV infection on the prognosis of patients with non-GCLS.

In addition to EBV infection, *Helicobacter pylori* (HP) infection is an important risk factor in the development

of gastric cancer. A previous study showed that EBV and HP coinfection may synergistically induce severe inflammatory responses in the stomach tissue and increase the risk of developing gastric cancer.[5] Regardless, it remains unclear whether the malignancy results from accumulated tissue damage caused by the EBV and HP coinfection or whether it is due to the close interaction between EBV and HP genes.[6] Although several studies found an association between the coinfection and gastric cancer pathogenesis, few studies have focused on the effect of an EBV and HP coinfection on the clinical outcomes and prognosis of gastric cancer.

Thus, we aimed to analyze the effect of EBV and HP coinfection on the clinicopathologic features and prognosis of gastric cancer, as well as to evaluate the role of EBV infection in non-GCLS.

MATERIALS AND METHODS

1. Patients

A total of 1,098 patients with gastric cancer underwent surgical resection at Asan Medical Center, a tertiary university hospital in Seoul, Korea, between September 2014 and August 2015. Of these, 142 patients who met the following criteria were excluded: (1) undergone endoscopic submucosal dissection previously, followed by an additional surgery due to non-curative resection (n=40); (2) could not analyze HP infection pathologically, because the normal tissue could not be acquired from operated gastric tissue (n=65); (3) could not analyze EBV infection pathologically for the same reason as above (n=25); (4) underwent palliative gastrectomy (n=4); and (5) underwent neoadjuvant chemotherapy (n=8). Finally, 956 patients were enrolled and were divided into groups, according to GCLS morphology, EBV infection, and HP infection (Fig. 1).

Clinicopathologic characteristics and oncologic outcomes were retrospectively analyzed. This study was approved by the Institutional Review Board of Asan Medical Center (IRB number: 2020-1272). The informed consent was waived because this design is a retrospective study.

2. Clinicopathologic definition

Clinical data, such as age, sex, and histopathologic results, including tumor size, tumor location, number of tumors, differentiation type, and presence of lymph node metastasis, were reviewed. Lymphovascular invasion was defined as the observable spread of tumor cells via lymphatic vessels. Perineural invasion (PNi) was defined as tumor cell infiltration in, around, and through the nerves.

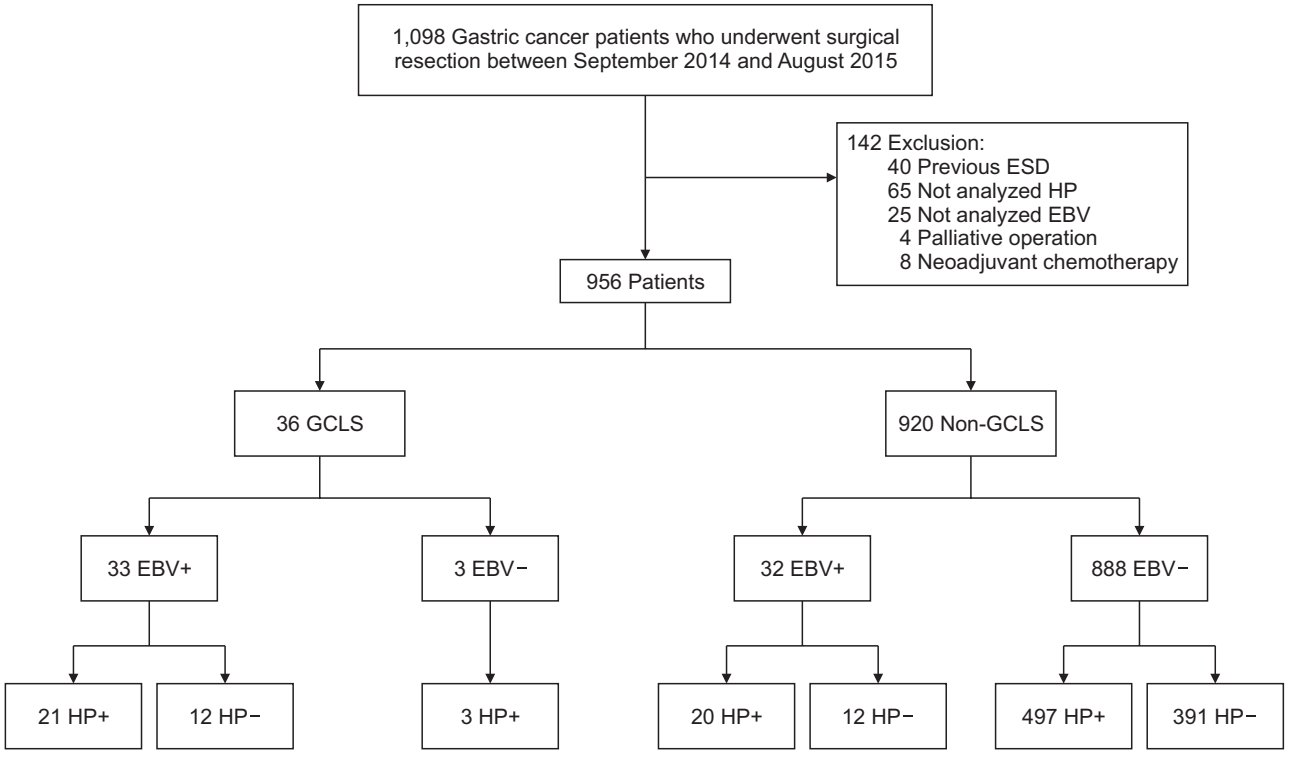

Fig. 1. Flowchart of the enrolled patients.
ESD, endoscopic submucosal dissection; HP, *Helicobacter pylori*; EBV, Epstein-Barr virus; GCLS, gastric carcinoma with lymphoid stroma.

The tumor stage was classified based on the 8th edition of the American Joint Committee on Cancer TNM staging system.[7] GCLS was defined according to the 2010 World Health Organization classification as a poor or undifferentiated lesion/tumor with prominent lymphoid infiltration.[8] After surgery, endoscopic examination and abdomen computed tomography were performed annually for 5 years. The follow-up period was defined as the interval from the operation day to the last outpatient clinic visit. The survival status of each patient was obtained from their medical records and the National Health Insurance Service records (South Korea).

3. Immunohistochemical staining and *in situ* hybridization

To identify HP, immunohistochemical (IHC) staining (1:500, rabbit polyclonal; CELL MARQUE, Rocklin, CA, USA) was performed on the non-tumoral gastric mucosa, more than 2 cm away from the carcinoma. In addition, one representative tumor section was selected, and IHC staining for p53 (1:1,000, mouse monoclonal; DAKO, Denmark, Glostrup) and human epidermal growth factor receptor 2 (HER2) (1:8; Mouse monoclonal, Ventana Medical Systems, Tucson, AZ, USA) as well as *in situ* hybridization (ISH) for EBV were performed.

All IHC staining was performed on 4-μm thick formalin-fixed paraffin-embedded sections, which were deparaffinized and re-hydrated using xylene and ethanol serially. Endogenous peroxidase was blocked by incubation in 3% H_2O_2 for 10 minutes, followed by heat-induced antigen retrieval. IHC labeling was performed using an autostainer (Benchmark XT; Ventana Medical Systems) and the OptiView DAB Detection Kit (Ventana Medical Systems), according to the manufacturer's protocol. EBV ISH was performed using an automated staining device (Benchmark XT; Ventana Medical Systems), according to the manufacturer's instruction. Briefly, 4-μm thick formalin-fixed paraffin-embedded sections were obtained with a microtome and dried at room temperature, followed by 20 minutes in an incubator at 65°C for 30 minutes. Sections were visualized with the Ventana EBER ISH iView Blue Detection Kit (Ventana Medical Systems) consisting of a cocktail of EBV-encoded small RNA probes. The intended target was the early RNA transcripts of EBV accumulated in the nuclei of EBV-infected cells, which are evaluated by a blue reaction that is localized to EBV-infected nuclei. All the immune-labeled slides and ISH slides were reviewed by two pathologists (J.Y.W and Y.S.P.).

HP was defined as the presence of any HP-like bacteria. The sample was judged as positive if any IHC-stained comma-shaped or S-shaped bacillus was 0.5 to 1.0 μm in width and 2.5 to 4.0 μm in length.[9] The p53 IHC staining and EBV ISH were classified into three categories according to the positive proportion and the pattern of expression: negative was less than 5%; equivocal was more than 5% and heterogeneously positive pattern; and overexpression was homogenously strong positive pattern.[10] HER2 protein expression was scored on a scale of zero to three using the recommendations of the gastric cancer consensus panel.[11]

4. Statistical analysis

Descriptive variables were summarized as mean±standard deviation. The differences in patient characteristics between the EBV+ and EBV– groups were compared using the independent t-tests and the chi-square test. Furthermore, the differences in patient characteristics according to EBV and HP infection were evaluated using an analysis of variance. Cox proportional hazards model was performed for univariate and multivariate analyses to determine the significant factors that affect overall survival (OS). The covariates that were significant in univariate Cox analysis were included in the multivariate evaluation. OS and disease-specific survival rates were calculated using the Kaplan-Meier method and compared using the log-rank test. Patients were censored at 5 years for OS if they were alive at 5 years after surgery. In addition, follow-up loss and recurrence were regarded as censoring. Patients alive and free of recurrence were censored at the last follow-up for relapse-free survival. A p-value of <0.05 indicated statistical significance. All statistical analyses were performed using the SPSS version 24 (IBM Corp., Armonk, NY, USA).

RESULTS

1. Clinicopathologic characteristics according to EBV and HP infection status

Table 1 summarizes the clinicopathologic characteristics according to EBV and HP infection status. The mean age was 58.6±11.9 years, and 63.6% of the patients were male. EBV and HP coinfection was found to be more associated with the male sex, proximal location, and GCLS morphology ($p<0.001$). The EBV and HP coinfection group tended to show equivocal p53 expression, while the other groups showed more negativity or overexpression of p53. HER2 expression was similar in all groups. The tumor stage, number of lesions, lymphovascular invasion, and PNi were not significantly different between the groups.

A subgroup analysis was performed by classifying the patients in more detail, such as EBV+/HP+ (4.3%), EBV+/HP– (2.9%), EBV–/HP+ (52.3%), and EBV–/HP– (40.5%) (Supplementary Table 1). Similarly, EBV infection was

Table 1. Comparison of the Clinicopathologic Characteristics between the EBV and HP Coinfection Group and the Other Groups (n=956)

Characteristics	EBV+/HP+ (n=41)	Others (n=915)	p-value
Age at diagnosis, yr	59.4±11.3	58.6±12.0	0.642
Male sex	36 (87.8)	575 (62.5)	0.001
Location of the tumor			<0.001
Upper third	23 (56.1)	139 (15.2)	
Middle third	11 (26.8)	284 (31.0)	
Lower third	7 (17.1)	492 (53.8)	
Lesions			0.194
Single	39 (95.1)	898 (98.1)	
Multiple	2 (4.9)	17 (1.9)	
Tumor size, cm	4.28±2.90	3.95±2.74	0.483
Pathologic T stage			0.723
T1	28 (68.3)	586 (64.0)	
T2	5 (12.2)	100 (10.9)	
T3&T4	8 (19.5)	229 (25.0)	
Pathologic N stage			0.768
N0	33 (80.5)	652 (71.3)	
N1	4 (9.8)	110 (12.0)	
N2	2 (4.9)	69 (7.5)	
N3	2 (4.9)	84 (9.2)	
Histology			<0.001
WD/MD	6 (14.6)	318 (34.8)	
PD/SRC	13 (31.7)	543 (59.3)	
GCLS	21 (51.2)	15 (1.6)	
Others*	1 (2.4)	39 (4.3)	
HER2			0.631
Score 0&1	33 (80.5)	776 (84.8)	
Score 2	4 (9.8)	75 (8.2)	
Score 3	4 (9.8)	64 (7.0)	
p53			<0.001
Negative	1 (2.4)	158 (17.3)	
Overexpression	4 (9.8)	236 (25.9)	
Equivocal	36 (87.8)	518 (56.8)	
AJCC stage			0.410
I	28 (68.3)	618 (67.5)	
II	9 (22.0)	149 (16.3)	
III	4 (9.8)	148 (16.2)	
LVi	11 (26.8)	292 (31.9)	0.494
PNi	6 (2.7)	220 (24.1)	0.164
Adjuvant chemotherapy	7 (2.9)	232 (25.4)	0.231
Follow-up period, mo	70.9±10.2	66.8±16.8	0.121

Data are presented as mean±SD or number (%).
EBV, Epstein-Barr virus; HP, *Helicobacter pylori*; T, tumor; N, node; WD, well differentiated; MD, moderate differentiated; PD, poorly differentiated; SRC, signet ring cell; GCLS, gastric carcinoma with lymphoid stroma; HER2, human epidermal growth factor receptor 2; AJCC, American Joint Committee on Cancer; LVi, lymphovascular invasion; PNi, perineural invasion.
*Others: mucinous adenocarcinoma, papillary adenocarcinoma, adenoneuroendocrine carcinoma.

found to be more associated with the male sex, proximal location, and GCLS morphology. However, these cases tended to show less p53 negativity or overexpression, regardless of HP infection status.

2. Risk factors related to OS in all patients with gastric cancer

Multivariate analysis revealed that age (hazard ratio [HR], 1.037; 95% confidence interval [CI], 1.023 to 1.051; p<0.001), tumor size (HR, 1.070; 95% CI, 1.015 to 1.128; p=0.012), and advanced American Joint Committee on Cancer stage (HR, 2.577; 95% CI, 1.525 to 4.356 for stage II and HR, 6.979; 95% CI, 4.086 to 11.912 for stage III; respectively, p<0.001) were independent predictors of OS (Table 2). Moreover, EBV infection (HR, 0.362; 95% CI, 0.131 to 0.996; p=0.049) and lower third location (HR, 0.624; 95% CI, 0.413 to 0.943; p=0.025) were inversely correlated with OS. However, EBV and HP coinfection was not significantly associated with OS (p=0.156).

3. Oncologic outcome according to EBV and HP infection status

The mean follow-up period after surgery was 66.9±16.5 months. The 5-year OS rate was 87.2%, and it was not significantly different between the EBV and HP coinfection group and other groups (97.6% vs 86.8%, log-rank p=0.144) (Fig. 2A). The 5-year disease-specific survival rate was 90.1%, and it was not significantly different between the EBV and HP coinfection group and other groups (95.1% vs 89.9%, log-rank p=0.305) (Fig. 2B). The subgroup analysis for OS was performed according to EBV and HP infection status (Supplementary Fig. 1). There were significant differences across the four groups in the following order: EBV+/HP–, EBV+/HP+, EBV–/HP+, and EBV–/HP– (97.6% vs 90.9% vs 88.0% vs 84.2%, respectively, log-rank p=0.040). In addition, OS was not significantly different between HP+ and HP– cases when EBV infection status was consistent (Supplementary Fig. 2).

4. Role of EBV infection in non-GCLS

The clinicopathologic characteristics between the EBV+ and EBV– groups in non-GCLS are summarized in Table 3. The EBV+ group showed a higher proportion of male sex (p=0.014) and proximal location of the tumor (p=0.001). The expression of p53 was significantly different between the two groups (p<0.001). Moreover, the overexpression or negative categories were more frequent in the EBV– group, while the EBV+ group tended to show p53 equivocal expression. HER2 expression was similar in both the EBV+ and EBV– groups. The tumor stage, number of lesions, lymphovascular invasion, and PNi were not significantly different between the two groups. In fact, the results for EBV+ and EBV– in all of the gastric cancer cases were similar to those in non-GCLS (Supplementary Table 2). Additionally, the EBV+ group showed a more frequent GCLS morphology than the EBV group for all types of gas-

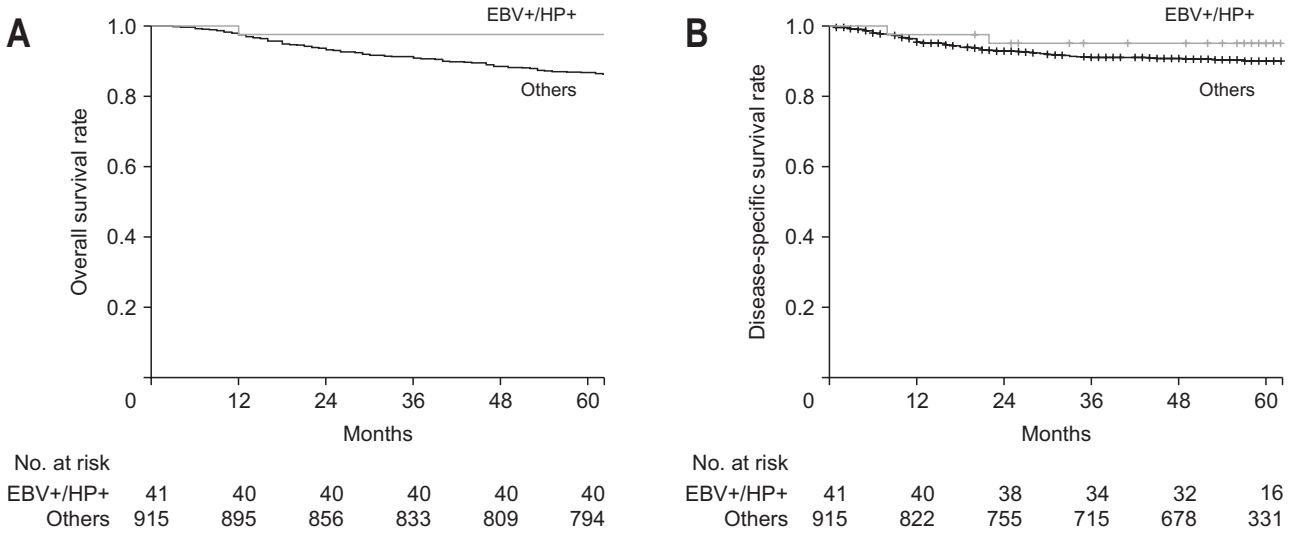

Fig. 2. Results of the survival analysis in all patients with gastric cancer, based on Epstein-Barr virus (EBV) and *Helicobacter pylori* (HP) infection status. (A) The 5-year overall survival rate (log-rank p=0.144) and (B) the 5-year disease-specific survival rate (log-rank p=0.305).

Table 2. Cox Proportional Hazards Model of the Factors Associated with Overall Survival in All Patients with Gastric Cancer (n=956)

Factor	Univariate analysis		Multivariate analysis*	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age	1.045 (1.030–1.060)	<0.001	1.037 (1.023–1.051)	<0.001
Sex (male)	1.478 (1.034–2.112)	0.032		
EBV and HP coinfection				
Others	1			
EBV+/HP+	0.437 (0.139–1.371)	0.156		
Histology				
Non-GCLS	1			
GCLS	0.334 (0.083–1.347)	0.123		
EBV infection	0.356 (0.132–0.963)	0.042	0.362 (0.131–0.996)	0.049
HP infection	0.772 (0.559–1.066)	0.116		
Tumor location				
Upper third	1		1	
Middle third	0.446 (0.288–0.692)	<0.001	0.775 (0.489–1.227)	0.277
Lower third	0.478 (0.325–0.702)	<0.001	0.624 (0.413–0.943)	0.025
Tumor size	1.202 (1.162–1.243)	<0.001	1.070 (1.015–1.128)	0.012
Number of lesions	0.325 (0.046–2.324)	0.263		
AJCC stage				
I	1		1	
II	3.860 (2.404–6.196)	<0.001	2.577 (1.525–4.356)	<0.001
III	13.057 (8.823–19.322)	<0.001	6.979 (4.086–11.912)	<0.001
LVi	4.764 (3.398–6.679)	<0.001		
PNi	4.685 (3.388–6.479)	<0.001	1.403 (0.934–2.106)	0.103

HR, hazard ratio; CI, confidence interval; EBV, Epstein-Barr virus; HP, *Helicobacter pylori*; GCLS, gastric carcinoma with lymphoid stroma; AJCC, American Joint Committee on Cancer; LVi, lymphovascular invasion; PNi, perineural invasion.
*Simultaneously, adjusted for age, sex, EBV infection, tumor location, tumor size, AJCC stage, LVi, and PNi.

tric cancer (p<0.001).

Multivariate analysis revealed that age (p<0.001), tumor size (p=0.003), advanced American Joint Committee on Cancer stage (p<0.001), and PNi (p=0.015) were independent predictors of OS (Table 4). Although EBV positivity did not result in a statistically significant survival difference in patients with non-GCLS (96.9% vs 86.4%, log-rank p=0.126) (Fig. 3A), better survival trends were found in the EBV+ group. This was because no patients died within 5 years in the EBV+ group. This trend was also similar to

Table 3. Comparison of the Clinicopathologic Characteristics between the EBV-Positive and EBV-Negative Groups in Non-GCLS (n=920)

Characteristics	EBV+ (n=32)	EBV– (n=888)	p-value
Age at diagnosis, yr	59.9±9.9	58.6±12.0	0.533
Male sex	27 (84.4)	550 (61.9)	0.014
Location of the tumor			0.001
Upper third	11 (34.4)	127 (14.3)	
Middle third	13 (40.6)	274 (30.9)	
Lower third	8 (25.0)	487 (54.8)	
Lesions			0.127
Single	30 (93.8)	872 (98.2)	
Multiple	2 (6.3)	16 (1.8)	
Tumor size, cm	3.57±2.19	3.98±2.76	0.404
Pathologic T stage			0.498
T1	23 (71.9)	566 (63.7)	
T2	4 (12.5)	97 (10.9)	
T3&T4	5 (15.6)	225 (25.3)	
Pathologic N stage			0.757
N0	26 (81.3)	628 (70.7)	
N1	2 (6.3)	109 (12.3)	
N2	2 (6.3)	68 (7.7)	
N3	2 (6.3)	83 (9.3)	
Histology			0.637
WD/MD	9 (28.1)	315 (35.5)	
PD/SRC	22 (68.8)	534 (60.1)	
Others*	1 (3.1)	39 (4.4)	
HP infection			0.477
HP (+)	20 (62.5)	497 (56.0)	
HP (–)	12 (37.5)	391 (44.0)	
HER2			0.414
Score 0 & 1	26 (81.3)	750 (84.5)	
Score 2	2 (6.3)	75 (8.4)	
Score 3	4 (12.5)	63 (7.1)	
p53			<0.001
Negative	0	158 (17.8)	
Overexpression	2 (6.3)	235 (26.5)	
Equivocal	30 (93.8)	495 (55.7)	
AJCC stage			0.529
I	24 (75.0)	597 (67.2)	
II	5 (15.6)	145 (16.3)	
III	3 (9.4)	146 (16.4)	
LVi	7 (21.9)	289 (32.5)	0.250
PNi	5 (15.6)	217 (24.5)	0.299
Adjuvant chemotherapy	6 (18.8)	227 (25.6)	0.420

Data are presented as mean±SD or number (%).
EBV, Epstein-Barr virus; GCLS, gastric carcinoma with lymphoid stroma; T, tumor; N, node; WD, well differentiated; MD, moderate differentiated; PD, poorly differentiated; SRC, signet ring cell; HP, *Helicobacter pylori*; HER2, human epidermal growth factor receptor 2; AJCC, American Joint Committee on Cancer; LVi, lymphovascular invasion; PNi, perineural invasion.
*Others: mucinous adenocarcinoma, papillary adenocarcinoma, adenoneuroendocrine carcinoma.

the disease-specific survival rate (96.0% vs 89.8%, log-rank p=0.199) (Fig. 3B).

DISCUSSION

In this study, EBV and HP coinfection was found to be significantly associated with the male sex, proximal location, and GCLS morphology, but it was not a significant predictor for OS. Regardless of HP infection, EBV infection status affected the clinicopathologic features of all types of gastric cancer. Although the OS rate was not significantly different between the EBV+ and EBV– groups in non-GCLS, better trends were observed in the EBV+ group.

HP is associated with neoplastic conditions such as gastric cancer and gastric mucosa-associated lymphoid tissue lymphoma. Moreover, EBV can induce oncogenesis in the host cell by activating various signaling pathways. The coinfection of these two pathogens may have synergic effects for inducing inflammation in stomach tissue and increasing the development of gastric cancer.[6,12-14] In addition to this mechanism, a gene product interaction may be oncogenic, specifically, in the presence of antigens to a cytotoxin-associated gene that is expressed by HP.[6,15] These findings support the results of our study that indicate that gastric cancer patients with EBV and HP coinfection showed similar clinicopathologic features to those previously reported with EBV+ gastric cancer. In addition, the coinfection was not a significant predictor of oncologic outcome. This suggests that EBV and HP coinfection is more likely to be associated with the development of gastric cancer, but it does not significantly affect the disease progress or prognosis.

EBV+ gastric cancer is reported in about 10% (1.3% to 20.1%) of all gastric cancer cases.[12] EBV is especially associated with GCLS, and it is very rare in non-GCLS. In our study, EBV+ was found in about 6.8% (65/956) of all gastric cancer cases, 91.7% (33/36) of GCLS cases, and 3.5% (32/920) of non-GCLS cases, similar to a previous report.[16] Moreover, a recent meta-analysis showed that EBV positivity is associated with improved survival in patients with gastric cancer.[17] The present study showed that the OS rate in non-GCLS cases was not significantly different between the EBV+ and EBV– groups. However, OS was significantly higher in the EBV+ group than in the EBV– group in all types of gastric cancer, including GCLS. A reason for this could be that the lymphoid stroma represents an antitumor effect to EBV-infected tumor cells by inducing the host's cellular and humoral immune responses, which is a significant factor related to the good prognosis of EBV+ gastric cancer.[18,19] Among early EBV+ gastric cancers, the histopathologic type with GCLS is associated with a very low risk of lymph node metastasis compared to other types of cancer; thus, it can be considered for endoscopic

Table 4. Cox Proportional Hazards Model of the Factors Associated with Overall Survival in Patients with Non-GCLS (n=920)

Factor	Univariate analysis		Multivariate analysis*	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age	1.047 (1.031–1.062)	<0.001	1.039 (1.025–1.054)	<0.001
Sex (male)	1.509 (1.054–2.159)	0.025		
EBV and HP coinfection				
Others	1			
EBV+/HP+	3.488 (0.488–24.929)	0.213		
EBV infection	0.353 (0.088–1.427)	0.144		
HP infection	0.763 (0.552–1.056)	0.102		
Tumor location				
Upper third	1			
Middle third	0.402 (0.258–0.627)	<0.001		
Lower third	0.422 (0.286–0.624)	<0.001		
Tumor size	1.200 (1.160–1.241)	<0.001	1.082 (1.026–1.141)	0.003
Number of lesions	0.336 (0.047–2.398)	0.277		
AJCC stage				
I	1		1	
II	3.929 (2.448–6.308)	<0.001	2.433 (1.437–4.120)	0.001
III	12.472 (8.414–18.487)	<0.001	6.165 (3.630–10.470)	<0.001
LVi	4.563 (3.251–6.406)	<0.001		
PNi	4.723 (3.406–6.549)	<0.001	1.631 (1.099–2.420)	0.015

GCLS, gastric carcinoma with lymphoid stroma; HR, hazard ratio; CI, confidence interval; EBV, Epstein-Barr virus; HP, *Helicobacter pylori*; AJCC, American Joint Committee on Cancer; LVi, lymphovascular invasion; PNi, perineural invasion.
*Simultaneously, adjusted for age, sex, EBV infection, tumor location, tumor size, AJCC stage, LVi, and PNi.

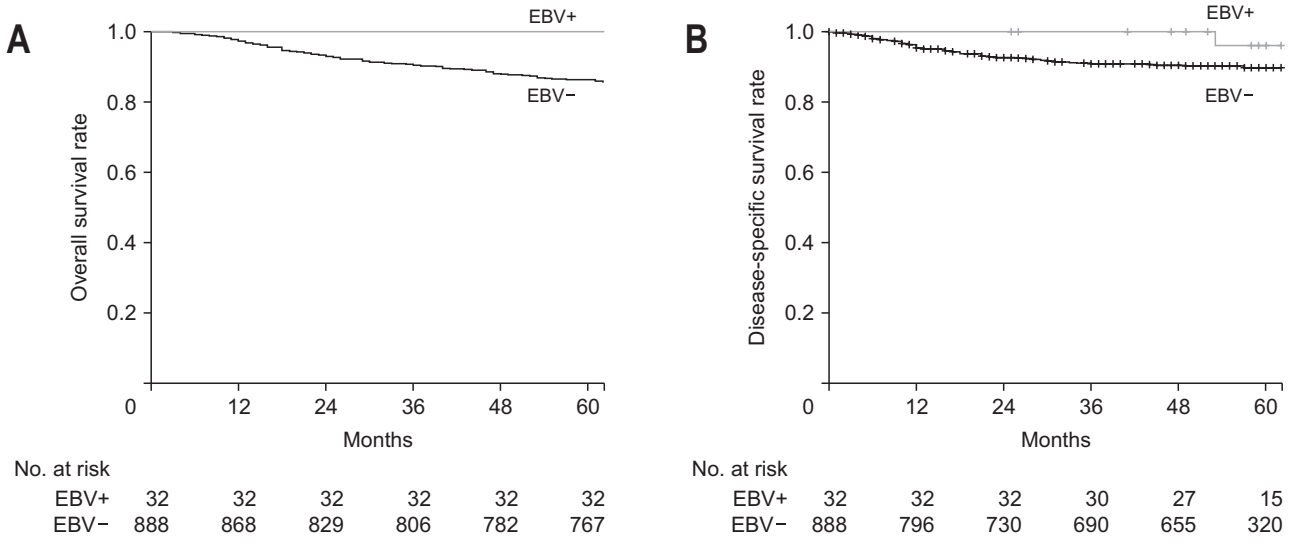

Fig. 3. Results of the survival analysis in patients with non-gastric carcinoma with lymphoid stroma, based on the Epstein-Barr virus (EBV) infection status. (A) The 5-year overall survival rate (log-rank p=0.126) and (B) the 5-year disease-specific survival rate (log-rank p=0.199).

resection even if there is submucosal infiltration.[20,21] Furthermore, in the case of advanced EBV+ gastric cancer, programmed death-ligand 1 (PD-L1) expression is high, and it is expected to respond to immune checkpoint inhibitor treatment.[12] Although GCLS is known to have a high association (>80% with ISH) with EBV positivity, it is not consistent.[22] Therefore, in patients with advanced gastric cancer including non-GCLS, ISH confirmation of EBV could be helpful for predicting their prognosis and treatment response better than histology alone.

Some studies have reported that HP+ gastric cancer has a better prognosis than gastric cancer without HP infection.[23] This could be related to the tumor-specific immune responses. As previously reported in EBV+ tumors, programmed death-ligand 1 expression has been found to be increased in HP-infected gastric epithelial cells, whereas

down-regulation of tumor-specific immune response is more frequently observed in patients without HP infection. Interestingly, the EBV–/HP– group showed the worst prognosis in our study, and we can speculate that tumors with less tumor-specific immune response are associated with a worse prognosis. However, to prove this, further research will be needed for tumor-infiltrating lymphocyte quantification using morphometry or IHC.

According to the Cancer Genome Atlas molecular subtype of gastric carcinoma, *TP53* mutation was rarely observed in EBV+ gastric carcinoma.[2] Similar to our study, p53 overexpression and p53 null-type expression were rarely observed in the EBV+ group. Instead, equivocal expression was predominant. Although studies on the correlation between p53 expression and EBV infection status are limited, some evidence was presented in a study by Taghavi *et al.* in 2010,[24] where p53 overexpression was frequently observed in esophageal squamous cell carcinoma with $p16^{INK4A}$ promoter hypermethylation. In fact, hypermethylation of $p16^{INK4A}$ was suspected to induce abnormal expression of the *MDM2/TP53* pathway.[24] Since most EBV+ gastric carcinoma is known to have promoter hypermethylation of $p16^{INK4A}$, predominant equivocal p53 expression in the EBV+ group could be similarly interpreted.[2]

There were several limitations to this study. First, selection bias may have occurred due to its single-center and retrospective study design. Second, the HP infection diagnostic tests, such as serum IgG antibody test, urea breath test, or rapid urease test, were not performed for all enrolled patients. Moreover, the serum pepsinogen level was only checked in few patients, and the gastric atrophy and metaplasia were not evaluated objectively. Therefore, we could not investigate the past infection of HP. Rather, we focused on the current HP infection by confirming it with IHC staining. Consequently, the results of the HP infection in our study have high specificity but low sensitivity. Third, some patients had a relatively short follow-up period. Since Asan Medical Center is a tertiary care hospital, patients are usually referred to a local hospital for routine evaluations after acute phase management. Nevertheless, this study has shown the association of EBV and HP infection with the clinical outcomes and prognosis of gastric cancer over a long-term follow-up period.

In conclusion, EBV and HP coinfection is not a significant prognostic factor for gastric carcinoma. Although the coinfection of two pathogens has been known to be related to carcinogenesis, it was not associated with the prognosis of gastric cancer after diagnosis in this study. Instead, regardless of HP infection, the EBV infection status affects the clinicopathologic features of all types gastric cancer. The EBV positivity also affects the clinicopathologic characteristics of patients with non-GCLS though it did not result in a statistically significant survival difference.

CONFLICTS OF INTEREST

J.Y.A. is an editorial board member of the journal but was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

AUTHOR CONTRIBUTIONS

Study concept and design: J.H.L., Y.S.P., I.S.L. Data acquisition: J.H.N., J.Y.S., J.H.L., Y.S.P., I.S.L., G.H.K., H.K.N., J.Y.A., K.W.J., D.H.K., K.D.C., H.J.S., G.H.L., H.Y.J. Data analysis and interpretation: J.H.N., J.Y.S. Drafting of the manuscript: J.H.N., J.Y.S. Critical revision of the article for intellectual content: J.H.L., Y.S.P., I.S.L. Approval of final manuscript: all authors.

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SUPPLEMENTARY MATERIALS

Supplementary materials can be accessed at https://doi.org/10.5009/gnl210593.

REFERENCES

1. Gomceli I, Demiriz B, Tez M. Gastric carcinogenesis. World J Gastroenterol 2012;18:5164-5170.

2. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. Nature 2014;513:202-209.
3. Song HJ, Srivastava A, Lee J, et al. Host inflammatory response predicts survival of patients with Epstein-Barr virus-associated gastric carcinoma. Gastroenterology 2010;139:84-92.
4. Lim H, Park YS, Lee JH, et al. Features of gastric carcinoma with lymphoid stroma associated with Epstein-Barr virus. Clin Gastroenterol Hepatol 2015;13:1738-1744.
5. Cárdenas-Mondragón MG, Torres J, Flores-Luna L, et al. Case–control study of Epstein–Barr virus and Helicobacter pylori serology in Latin American patients with gastric disease. Br J Cancer 2015;112:1866-1873.
6. Singh S, Jha HC. Status of Epstein-Barr virus coinfection with Helicobacter pylori in gastric cancer. J Oncol 2017;2017:3456264.
7. Rice TW, Patil DT, Blackstone EH. 8th edition AJCC/UICC staging of cancers of the esophagus and esophagogastric junction: application to clinical practice. Ann Cardiothorac Surg 2017;6:119-130.
8. Lauwers GY, Carneiro F, Graham DY. Gastric carcinoma. In: Bosman FT, Carneiro F, Hruban RH, Theise ND, eds. WHO Classification of Tumours of the Digestive System. 4th ed. Lyon: International Agency for Research on Cancer, 2010:48-58.
9. Lee JY, Kim N. Diagnosis of Helicobacter pylori by invasive test: histology. Ann Transl Med 2015;3:10.
10. McCluggage WG, Soslow RA, Gilks CB. Patterns of p53 immunoreactivity in endometrial carcinomas: 'all or nothing' staining is of importance. Histopathology 2011;59:786-788.
11. Rüschoff J, Dietel M, Baretton G, et al. HER2 diagnostics in gastric cancer-guideline validation and development of standardized immunohistochemical testing. Virchows Arch 2010;457:299-307.
12. Naseem M, Barzi A, Brezden-Masley C, et al. Outlooks on Epstein-Barr virus associated gastric cancer. Cancer Treat Rev 2018;66:15-22.
13. Dávila-Collado R, Jarquín-Durán O, Dong LT, Espinoza JL. Epstein-Barr virus and Helicobacter pylori co-infection in non-malignant gastroduodenal disorders. Pathogens 2020;9:104.
14. Matsusaka K, Funata S, Fukayama M, Kaneda A. DNA methylation in gastric cancer, related to Helicobacter pylori and Epstein-Barr virus. World J Gastroenterol 2014;20:3916-3926.
15. Cárdenas-Mondragón MG, Carreón-Talavera R, Camorlinga-Ponce M, Gomez-Delgado A, Torres J, Fuentes-Pananá EM. Epstein Barr virus and Helicobacter pylori co-infection are positively associated with severe gastritis in pediatric patients. PLoS One 2013;8:e62850.
16. Cho J, Kang MS, Kim KM. Epstein-Barr virus-associated gastric carcinoma and specific features of the accompanying immune response. J Gastric Cancer 2016;16:1-7.
17. Camargo MC, Kim WH, Chiaravalli AM, et al. Improved survival of gastric cancer with tumour Epstein-Barr virus positivity: an international pooled analysis. Gut 2014;63:236-243.
18. Watanabe H, Enjoji M, Imai T. Gastric carcinoma with lymphoid stroma. Its morphologic characteristics and prognostic correlations. Cancer 1976;38:232-243.
19. Luo Y, Liu Y, Wang C, Gan R. Signaling pathways of EBV-induced oncogenesis. Cancer Cell Int 2021;21:93.
20. Lim H, Lee IS, Lee JH, et al. Clinical application of early gastric carcinoma with lymphoid stroma based on lymph node metastasis status. Gastric Cancer 2017;20:793-801.
21. Shin DH, Kim GH, Lee BE, et al. Clinicopathologic features of early gastric carcinoma with lymphoid stroma and feasibility of endoscopic submucosal dissection. Surg Endosc 2017;31:4156-4164.
22. WHO Classification of Tumours Editorial Board. Digestive system tumours. 5th ed. Lyon: International Agency for Research on Cancer, 2019.
23. Fang WL, Huang KH, Chang SC, et al. Comparison of the clinicopathological characteristics and genetic alterations between patients with gastric cancer with or without Helicobacter pylori infection. Oncologist 2019;24:e845-e853.
24. Taghavi N, Biramijamal F, Sotoudeh M, et al. p16INK4a hypermethylation and p53, p16 and MDM2 protein expression in esophageal squamous cell carcinoma. BMC Cancer 2010;10:138.