# *Epstein–Barr virus infection in patients with chronic gastritis without* Helicobacter pylori *infection*

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## ABSTRACT

***Background/Aims:*** *The association of Epstein–Barr virus (EBV) with gastric malignancies has been proven by many studies in the literature. However, information about EBV-associated inflammation/gastritis remains limited. The aim of this study is to establish the prevalence of latent EBV infection in patients with chronic gastritis without* H. pylori *infection.*

***Materials and Methods:*** *In this study, 119 patients with gastritis without* H. pylori *infection were included. Furthermore, 28 patients with* H. pylori *gastritis were included in the study as a control group. Chromogenic in situ hybridization (EBV-encoded RNA) and immunohistochemistry (LMP-1 antibody) were performed in all 147 cases. The prevalence of EBV and its relationship with age, sex, the affected part of the stomach, the density of inflammation, inflammatory activity, intestinal metaplasia, and atrophy were analyzed.*

***Results:*** *In this study, 14 cases showed positive immunostaining for EBV. EBV positivity was seen mostly in the lymphoid tissue (13 cases), but it was also detected at the gastric epithelium (7 cases). The mean age of the patients was 44 years, which was slightly younger than that of the EBV-negative cases (48 years). The inflammation density was higher in EBV-positive cases than the EBV-negative gastritis cases (p=0.002). Intestinal metaplasia was detected in 7% of the cases. EBV-positive cases had a higher incidence of atrophy without intestinal metaplasia (21% vs 3.8% without EBV).*

***Conclusion:*** *EBV was detected in 12% of the cases with gastritis without* H. pylori *infection. Endoscopic follow-up may be appropriate for patients with gastritis, who have atrophy without intestinal metaplasia and are* H. pylori *negative but EBV positive.*

***Keywords:*** *EBV, prevalence, gastritis, atrophy,* Helicobacter pylori

## INTRODUCTION

Epstein-Barr virus (EBV) is a ubiquitous gamma herpes virus that causes persistent infection in over 90% of the world's population (1). EBV can cause both lytic and latent infection. During lytic infection, there is a production of virus, lysis of the host cells, and a broad expression of viral proteins. After primary infection, latency is established in resting memory B-lymphocytes, where only a small portion of the viral genome is expressed. Latent infection can also reactivate, allowing the virus to spread. The relationships between EBV and autoimmune diseases such as rheumatoid arthritis, Sjögren's syndrome, and systemic lupus erythematosus have been shown in some previous studies (2). Active chronic EBV infection in different parts of the body has also been well defined (3). However, there are no studies about its relationship with chronic gastritis in the current literature.

The association of EBV with gastric carcinoma (4-6) and gastric lymphoma is well described; however, the association of EBV with gastritis is underrecognized. Unique case reports of patients with acute gastritis (7-10) and peptic ulcer disease secondary to the lytic phase of EBV infection were published before. Moreover, the few studies that do exist about latent EBV infection and chronic gastritis are mostly polymerase chain reaction (PCR) based, thus being open to virion contaminants (11-15). Only a small number of cases were used for in situ hybridization (15, 16). Furthermore, in most of the studies, chronic atrophic gastritis cases, *H. pylori* gastritis cases (11, 17), or cases with normal gastric mucosa were included (18).

The prevalence of *H. pylori* in developing countries ranges from 60% to 85% (19). The accepted causes of *H. pylori*-negative chronic gastritis with moderate and severe

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lymphoid inflammation are mostly inflammatory bowel diseases or autoimmune gastritis (19). Infectious causes, except *H. pylori*, are not well documented. There is no evidence about the prevalence of EBV infection in *H. pylori*-negative gastritis in the literature.

The aim of this study is to establish the prevalence of latent EBV infection in patients with chronic gastritis without *H. pylori* infection.

## MATERIALS AND METHODS

### Inclusion and exclusion criteria of the study group

Endoscopic biopsies from 941 gastritis cases of antral gastritis without *H. pylori* were searched and identified from the hospital's database. All the cases without *H. pylori* were reanalyzed. All the cases with known/suspected etiology (chemical gastropathy, chronic atrophic gastritis, etc.) and cases with previous *H. pylori* therapy were excluded. The tissues without tissue Giemsa stain for *H. pylori* were also excluded. In Giemsa stains of 12 cases, *H. pylori* was suspected and immunohistochemical *H. pylori* stain was applied. Cases with positive immunohistochemical staining were excluded.

### Histopathological evaluation

Endoscopic biopsies from 119 cases were included to the study. Twenty-eight *H. pylori* positive cases, which were randomly chosen from the electronic database, were also included in the study as a control group.

All the cases were evaluated with the updated Sydney classification (20), and the Wotherspoon Grading System was used for lymphoid infiltration (21). Activity, intestinal metaplasia, and atrophy were classified based on the updated Sydney classification; Sydney and Wotherspoon grading were both used for evaluating inflammation. Additionally, intraepithelial lymphocytosis (≥25 lymphocyte/100 epithelial cells) and the presence of Mott bodies were also evaluated. All biopsies included in the study were endoscopic and were taken from the gastric antrum. All histological parameters were evaluated both in the study and control groups.

### Immunohistochemistry

Gastric tissue sections were deparaffinized and immunostained with the LMP-1 antibody (clone CS1, CS2, CS3, and CS4) using an automated system (Bond Max, Leica Biosystems, Germany). Cytoplasmic staining of more than 5% of the cells was accepted as positive staining. Epithelium and lymphoid tissue were evaluated separately. The LMP-1 antibody was applied to all *H. pylori*-negative tissues and 28 *H. pylori*-positive tissues.

### In situ hybridization

The EBV status of the samples with gastritis was determined by EBV-encoded RNA (EBER) chromogenic in situ hybridization by using sections of paraffin-embedded materials. The experiments were performed according to the manufacturers' instructions for an automated system (Bond Max). Appropriate positive and negative controls were included in all analyses. Nuclear staining in more than 5 cells was accepted as positive. Epithelium and lymphoid tissue were evaluated separately. EBV status was evaluated in all *H. pylori*-negative tissues and 28 *H. pylori*-positive tissues.

### Statistical analysis

Statistical analysis was performed using the Statistical Packages for the Social Sciences (SPSS) for Windows, version 22.0 (IBM Corp., Armonk, NY, USA) data analysis program. The Pearson chi-square test was used for comparing proportions among groups, and the Fisher exact test was used when appropriate. A $p<0.05$ was accepted as significant.

## RESULTS

### Clinical findings

The mean age of the EBV-positive/*H. pylori*-negative cases (Group 1-GR1) was 44 years (range, 26 to 81 years), that of

**Table 1.** Demographic characteristics of the study groups.

| | Gender, n (%) | | |
|---|---|---|---|
| Group | Male | Female | Total |
| EBV-positive *H. pylori*-negative (GR1) | 2 (14) | 12 (86) | 14 |
| EBV-negative *H. pylori*-negative (GR2) | 45 (43) | 60 (57) | 105 |
| EBV-negative *H. pylori*-positive (GR3) | 15 (42) | 13 (53) | 28 |

p=0.050.
EBV: Epstein–Barr virus; GR1: Group 1; GR2: Group 2; GR3: Group 3; n: number.

### MAIN POINTS

- *The association of EBV with gastric carcinoma and gastric lymphoma is well described; however, the association of EBV with gastritis is underrecognized.*
- *EBV prevalence in H. pylori-negative gastritis was found to be 12%.*
- *EBER in situ hybridization was more useful in detecting these cases.*

**Table 2.** Wotherspoon grading of the study groups.

| | Grade, n (%) | | | |
|---|---|---|---|---|
| Group | 1 | 2 | 3 | Total |
| EBV-positive *H. pylori*-negative (GR1) | 0 (0) | 11 (79) | 3 (21) | 14 |
| EBV-negative *H. pylori*-negative (GR2) | 24 (23) | 77 (73) | 4 (4) | 105 |
| EBV-negative *H. pylori*-positive (GR3) | 11 (39) | 17 (61) | 0 (0) | 28 |

p=0.002.
EBV: Epstein–Barr virus; GR1: Group 1; GR2: Group 2; GR3: Group 3; n: number.

**Table 3.** Activation score of the study groups.

| | Activation, n (%) | | | | |
|---|---|---|---|---|---|
| Group | 0 | 1 | 2 | 3 | Total |
| EBV-positive *H. pylori*-Negative (GR1) | 0 (0%) | 3 (21%) | 10 (72%) | 1 (7%) | 14 |
| EBV-negative *H. pylori*-Negative (GR2) | 11 (11%) | 30 (29%) | 61 (58%) | 3 (3%) | 105 |
| EBV-negative *H. pylori*-Positive (GR3) | 0 (%0) | 2 (7%) | 23 (82%) | 3 (11%) | 28 |

p=0.003.
EBV: Epstein–Barr virus; GR1: Group 1; GR2: Group 2; GR3: Group 3; n: number.

the EBV/*H. pylori*-negative cases (Group 2-GR2) was 48 years, and that of *H. pylori*-positive/EBV-negative cases (Group 3-GR3) was 45 years. There was a significant predominance of female cases in GR1, with 12 women and 2 men (F/M:6 vs GR2:1,3 vs GR3:0,9) (p=0.05) (Table 1).

### *EBV status*

EBV positivity was defined as LMP-1 and/or EBER positivity. Among the patients, 14 showed positivity for EBV (12%). EBV positivity was seen mostly in the lymphoid tissue (13 cases), but it was also detected at the gastric epithelium (7 cases). Only one case showed both LMP-1 and EBER positivity. LMP-1 positivity was seen in only 3 cases (2 both in the epithelium and lymphoid tissue, 1 only in the lymphoid tissue) (Figure 1). Twelve cases had EBER positivity in the lymphoid tissue and four of them also had positivity in the epithelium (Figures 2 and 3).

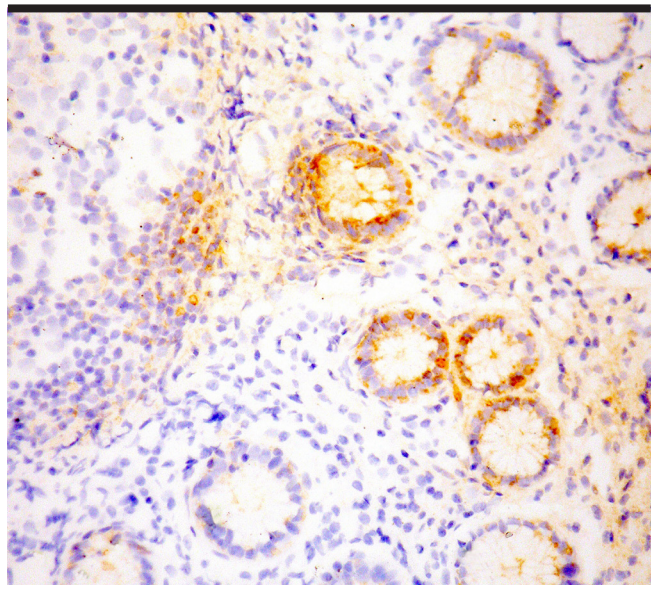

**Figure 1.** Cytoplasmic LMP-1 positivity both in the gastric epithelium and in the lymphoid tissue. LMP-1 positivity was seen only in 3 cases.

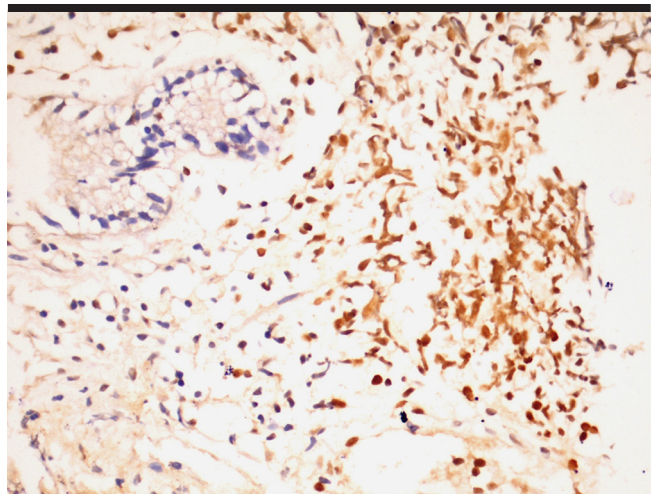

**Figure 2.** EBER positivity in the lymphoid tissue. Nuclear EBER positivity was seen in 12 cases in the lymphoid tissue.

None of the cases had positivity only in the epithelium. Background staining was detected in immunohistochemistry in 10 cases and in situ hybridization in 7 cases. Staining was repeated in these biopsies, and if the background staining persisted, the case was accepted as negative. There was no positivity in the control group.

### *Histopathological findings*

The mean updated Sydney inflammation grade was 2.2 for GR1, GR2, and GR3. There was no difference in Syd-

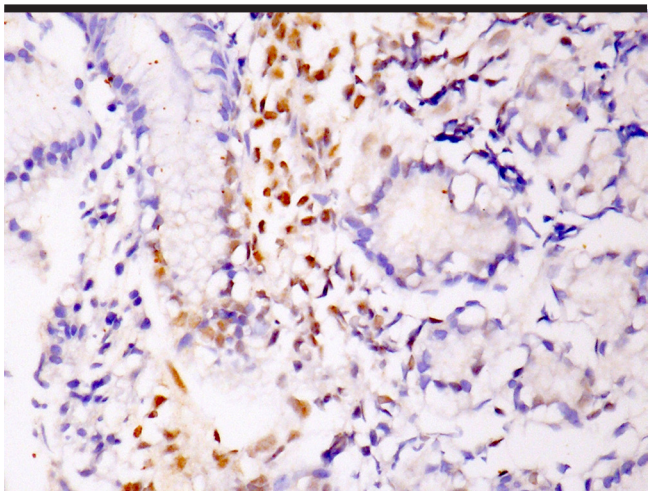

**Figure 3.** Nuclear EBER positivity both in the gastric epithelium and lymphoid tissue. EBER positivity was seen both in the epithelium and lymphoid tissue in 4 cases.

ney inflammation grades between the groups. The results of the Wotherspoon grading were quite different. All GR1 cases were grade 2 (79%) and grade 3 (21%). However, in the other groups, grade 1 cases occurred (23% in GR2 and 40% in GR3), and grade 3 cases were less common (4% in GR2 and no cases in GR3) (p=0.002) (Table 2). The grade 2 and 3 activation score is less common in GR1 compared to that in GR3 (Table 3). Only one case showed intestinal metaplasia in GR1 versus 10 cases in GR2. Atrophy was present in 3 cases in GR1 (21%), 4 cases in GR2 (4%), and no case in GR3. Four cases could not be evaluated for atrophy due to inflammation. Intraepithelial lymphocytosis and Mott bodies were not seen in any of the cases.

## DISCUSSION

Infectious causes of chronic gastritis other than *H. pylori* are not well documented in the literature. However, it is known that in addition to *H. pylori*, which is the most common agent of gastric carcinoma pathogenesis, EBV is also an important factor (22). EBV is the cause of 10% of gastric carcinomas (23). This is thought to be due to its latent presence in both the epithelium and lymphoid tissues (24).

There are case reports in the literature on gastritis caused by the lytic phase of EBV. These cases mostly cause acute infection (7-10). In recent years, studies have been conducted on EBV latent infection in the gastrointestinal mucosa. These studies were mostly performed using PCR (11-15). Latent infection in the tissue was investigated only in a small number of cases (15). No previous studies have investigated the presence of EBV in *H. pylori*-negative cases.

In our study, we investigated the presence of EBV by immunohistochemical and in situ hybridization methods, which are frequently used routine methods, in 119 *H. pylori*-negative gastritis cases. Thus, we planned to reveal the prevalence of EBV in *H. pylori*-negative chronic gastritis cases. By choosing the methods that can be used easily in many centers, we planned to make the results reproducible at the end of the study. In *H. pylori* negative gastritis cases, we found the prevalence of EBV as 12%. This rate was less than that observed in a study using PCR analysis (46%) (15) but higher than that in another study using the immunohistochemical and in situ hybridization methods in the tissue (5%) (16). However, since previous studies were conducted with either *H. pylori*-positive or *H. pylori* status unknown cases, it was not reliable to compare this rate with that found in other studies.

Given the demographic characteristics of the patients, we did not find a significant difference between the groups in terms of age. The average age was 44 years in GR1, 45 years in GR2, and 48 years in GR3. Interestingly, there was a female predominance in GR1. This was, in fact, a finding incompatible with EBV-positive gastric cancers, which showed male predominance. However, we think that this can be explained by the fact that gastric cancer is more frequent in men (22). Multivariate statistical analysis and further studies are required to prove this thesis.

Histopathologically, when evaluated according to the updated Sydney scoring system, the inflammatory score was found to be similar to that of other chronic gastritis cases. On the other hand, with detailed Wotherspoon grading, the inflammation is higher in EBV-positive gastritis. Furthermore, most of the patients had moderate activity, but it was less than that of patients with *H. pylori* gastritis. The intestinal metaplasia rate was slightly lower than that in other groups (7% vs 9%). On the contrary, atrophy was present at a much higher rate than that that in other groups (21% vs 4%). Intestinal metaplasia and atrophy are however seen together in classical *H. pylori* gastritis. According to some authors, atrophy without intestinal metaplasia is a very rare finding (25). Interestingly, in EBV-positive/*H. pylori*-negative cases of gastritis, atrophy without intestinal metaplasia was frequently observed. It is also mentioned in the literature that in EBV-positive gastric carcinoma, the virus is localized in

the atrophic epithelium (23). This can be a specific feature of EBV gastritis.

One study reported that intraepithelial lymphocytosis was more prevalent in EBV infections (26). For this reason, we evaluated intraepithelial lymphocytosis additionally. Further, Mott cells were described in gastric cancers with EBV positivity (27). Thus, we also investigated the presence of Mott cells. We did not find intraepithelial lymphocytosis and Mott cells in any of the cases.

In conclusion, EBV prevalence in *H. pylori*-negative gastritis was found to be 12%. Among our methods, EBER in situ hybridization was more useful in detecting these cases. EBV positivity was not recorded in 28 *H. pylori*-positive cases. However, since EBER-negative cases can be immunohistochemically LMP-1 positive, we think that it is more helpful to use both methods together. EBV positivity was found to be higher in women and middle-aged patients. Atrophy without intestinal metaplasia was much more frequent than in other gastritis. The findings of the study suggest that it will be good practice to search for EBV when atrophy without intestinal metaplasia is detected in gastric mucosa in *H. pylori*-negative middle-aged female patients. Since the effect of EBV on gastric carcinogenesis is known, we also think that follow-up and treatment protocols should be developed for these cases.

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***Author contributions:*** Concept - N.D., E.H., O.O.; Design - N.D., E.H., O.O.; Supervision - N.D.; Resource - N.D., E.H., O.O.; Materials - N.D., E.H., O.O., C.L., E.P.; Data Collection and/or Processing - N.D., E.H., O.O., C.L., E.P.; Analysis and/or Interpretation - N.D., E.H., O.O., C.L., E.P.; Literature Search - N.D., E.H., O.O., C.L., E.P.; Writing - N.D., E.H., O.O.; Critical Reviews - C.L., E.P.

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