

Epstein-Barr virus infection and gastric carcinoma in São Paulo State, Brazil

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Abstract

Epstein-Barr virus (EBV) is a ubiquitous herpesvirus, and most people have serological evidence of previous viral infection at adult age. EBV is associated with infectious mononucleosis and human cancers, including some lymphomas and gastric carcinomas. Although EBV was first reported in lymphoepithelioma-like gastric carcinoma, the virus was also found in conventional adenocarcinomas. In the present study, 53 gastric carcinomas diagnosed in São Paulo State, Brazil, were evaluated for EBV infection by non-isotopic *in situ* hybridization with a biotinylated probe (Biotin-AGACACCGTCCTCACCACCCGGGACTTGTA) directed to the viral transcript EBER-I, which is actively expressed in EBV latently infected cells. EBV infection was found in 6 of 53 (11.32%) gastric carcinomas, mostly from male patients (66.7%), with a mean age of 59 years old. Most EBV-positive tumors were in gastric antrum. Two EBV-positive tumors (33.3%) were conventional adenocarcinomas, whereas four (66.7%) were classified as lymphoepithelioma-like carcinomas. EBV infection in gastric carcinomas was reported elsewhere in frequencies that range from 5.6% (Korea) up to 18% (Germany). In Brazil, a previous work found EBV infection in 4 of 80 (5%) gastric carcinomas, whereas another study found 4.7 and 11.2% of EBV-positive gastric carcinomas of Brazilians of Japanese origin or not, respectively. In the present study, the frequency of EBV-positive gastric carcinomas is similar to that reported in other series, and the clinicopathologic characteristics of these EBV-positive tumors are in agreement with the data in the literature.

Key words

- Epstein-Barr virus
- Gastric carcinoma
- *In situ* hybridization
- Brazil

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Introduction

Epstein-Barr virus (EBV) has been associated with a variety of lymphoid and epithelial malignancies, such as Burkitt's lymphoma (1,2), Hodgkin's disease (3,4), T-cell lym-

phomas (5,6), AIDS-related lymphomas (7, 8), nasopharyngeal carcinoma (1,9,10), and lymphoepithelioma-like carcinomas (LELC) of several organs including salivary glands (11), thymus (12,13), and lung (14).

Early in the 1990s, some papers reported

the association between gastric carcinoma and EBV infection. Burke et al. (15) first detected EBV in a LELC of the stomach by the polymerase chain reaction, and other investigators also confirmed this finding (16-21). LELC is an uncommon type of gastric carcinoma (17,19,22) strongly associated with EBV, with this association ranging from 77.8 to 87.5% of cases (17,19-21). Occurrence in elderly patients, male predominance, gastric cardia and middle portion involvement in most cases, and high survival rates were described in association with gastric LELC (17,19,20,22).

In 1991, Shibata et al. (17) first presented a case of EBV-positive conventional gastric carcinoma. Several investigators subsequently added new information regarding the prevalence and clinicopathologic characteristics of EBV infection in gastric carcinomas of different populations from North, Central and South America, Europe and Asia; however, there are few reports concerning clinicopathologic data of EBV-related gastric carcinomas among Brazilians, including one study that compares characteristics of EBV-positive gastric carcinomas from Brazilians of Japanese origin or not (23).

Thus, the aim of the present study was to provide additional information about the prevalence and characteristics of EBV infection in gastric carcinomas diagnosed in São Paulo State, Brazil, and compare the results with other series of EBV-positive gastric cancer elsewhere.

Material and Methods

Case selection

Fifty-three gastrectomy specimens with gastric carcinomas diagnosed in the Department of Pathology, State University of São Paulo (Botucatu, SP, Brazil), were included in this study. Representative formalin-fixed paraffin-embedded blocks from the tumor, surrounding non-neoplastic gastric mucosa,

and regional lymph nodes with metastases were selected. Gastric carcinomas were classified according to the Lauren's classification. The undifferentiated and lymphoepithelioma-like types of carcinoma were also considered for classification, and the criteria for diagnosis of LELC were applied as described by Watanabe et al. (22). Clinicopathologic data were obtained from pathology reports, including patient age and sex as well as the anatomical site of the tumor (cardia, middle portion or antrum) and the histologic type of carcinoma.

All cases were confirmed as carcinomas with the demonstration of keratin reactivity by immunohistochemistry. Of the 53 patients studied, 35 were males and 17 were females. The gender of one patient was not available. Mean age was 58.8 years, ranging from 30 to 80 years.

In situ hybridization

EBV infection was assessed by RNA *in situ* hybridization with a 30-bp biotinylated probe (Biotin-AGACACCGTCCTCACCAC CCGGGACTTGTA) for RNA EBER-I, an EBV transcript actively expressed in latently infected cells. Briefly, tissue sections were placed on poly-D-lysine-treated glass slides and incubated at 70°C for one day. After the slides were routinely deparaffinized, endogenous peroxidase was blocked with 3% H₂O₂ solution. Enzymatic digestion was performed with proteinase K (0.02 µg/µl final concentration) and the tissues were then dehydrated in ethanol and dried at room temperature. The slides were incubated in prehybridization solution (20 mM sodium phosphate/Denhardt's solution 1X/0.1% dextran sulfate) for 60 min at 37°C. After the addition of the EBER-I probe (0.25 ng/ml final concentration), tissues were incubated overnight at 37°C in a humid chamber. The signal was amplified with the ABC Elite kit (Vector Corp., Burlingame, CA, USA), and the reaction was developed with 3,3'-diaminobenzi-

dine (Sigma, St. Louis, MO, USA) solution. The slides were counterstained with methyl green and mounted with Permount® resin (Fischer Chemicals Inc., Fair Law, NJ, USA). A case of nasopharynx LELC previously known to be EBV positive was used as positive control. The cells presenting a positive signal for EBV EBER-I RNA had dark brown to black nuclear staining.

Results

EBER-I was positive in 6 of the 53 cases (11.3%). Five of the 6 positive cases (83.3%) were male patients, with a mean age of 59 years, ranging from 52 to 67 years. The only female patient with an EBER-I-positive gastric carcinoma was 65 years old.

Pathologic and *in situ* hybridization findings

According to the anatomical site of the gastric carcinoma, 22 (41.5%) carcinomas were present in the antrum, 6 (11.3%) in the middle portion of the stomach, and 5 (9.4%) in the cardia. Unfortunately, information regarding the anatomical site was unavailable for 3 EBV-positive cases. Two (33.3%) of the 6 EBV-positive cases were localized in the antrum and one (16.6%) in the middle portion of the stomach.

The distribution of the histological types of carcinomas (including EBV-positive cases) is shown in Table 1. Among the 6 EBV-positive carcinomas, 2 (33.3%) were conventional carcinomas of tubular (Figure 1A) and undifferentiated types (Figure 1E), and 4 (66.7%) were LELC (Figure 1C). The EBER-I signal was uniformly distributed in the nuclei of carcinoma cells in all 6 positive cases (Figure 1B,D,F). The surrounding lymphocytes and non-neoplastic gastric mucosa with chronic gastritis or intestinal metaplasia were EBV negative. It is worth noting that EBV sequences were also detected in a case with metastatic carcinoma in regional lymph nodes. This finding indicates that the

metastatic gastric carcinoma recapitulates the EBV infection in the neoplastic cells from the primary tumor.

Table 1. Distribution of gastric carcinomas in Brazilian patients by histologic type.

Histologic types	Total	EBER-I-positive cases
Intestinal (including tubular adenocarcinoma)	27 (51.0%)	1 (37%)
Diffuse	18 (34%)	- (0%)
Undifferentiated	1 (1.8%)	1 (100%)
LELC	7 (13.2%)	4 (57.1%)
Total	53 (100%)	6 (11.3%)

LELC, lymphoepithelioma-like carcinoma.

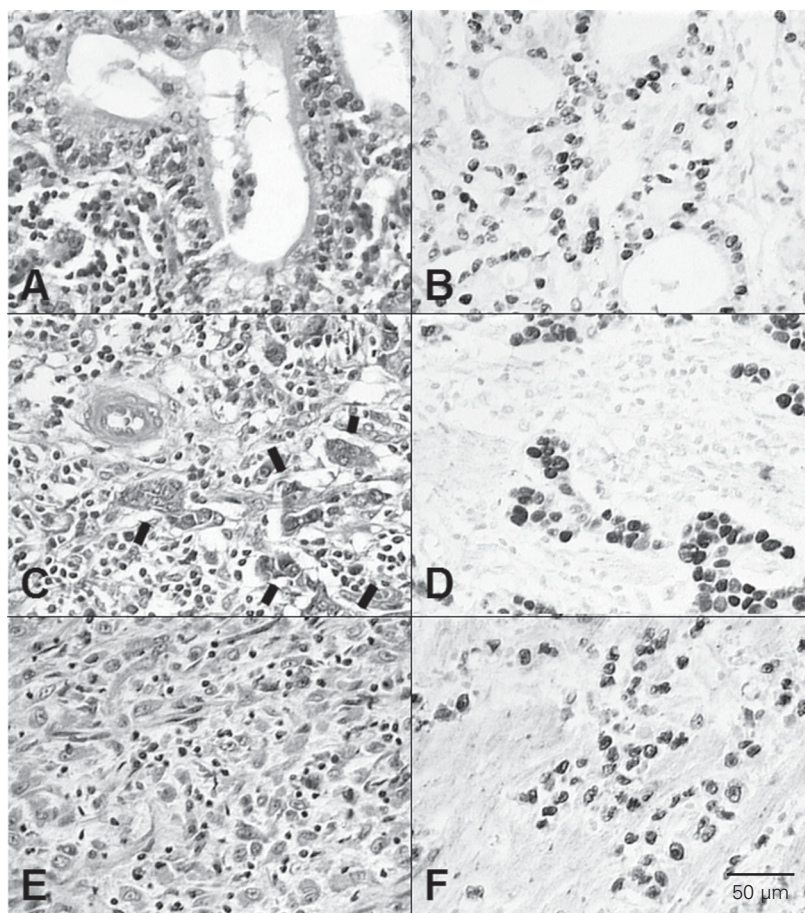


Figure 1. Photomicrographs of the Epstein-Barr virus-positive carcinomas. A, Tubular adenocarcinoma (hematoxylin and eosin, 400X). B, Tubular adenocarcinoma showing nuclear EBER-I signals (EBER-I, 400X). C, Lymphoepithelioma-like carcinoma (hematoxylin and eosin, 400X). D, Lymphoepithelioma-like carcinoma showing nuclear EBER-I signals (EBER-I, 400X). E, Undifferentiated carcinoma (hematoxylin and eosin, 400X). F, Undifferentiated carcinoma showing nuclear EBER-I signals (EBER-I, 400X).

Discussion

The Brazilian National Cancer Institute (Instituto Nacional do Câncer, INCA) reports 20,000 new cases of gastric cancer yearly, and it is believed that about 11,000 patients die because of the disease (24). Diet, smoking, alcohol intake, previous gastric surgery, and some gastric diseases, such as pernicious anemia, gastritis and *Helicobacter pylori* infection, are considered to be risk factors for the development of cancer. The association between EBV and gastric cancer was first detected by Burke et al. (15), and many reports showing this strong association have been published since then.

The frequency of EBV-positive gastric carcinomas can reach up to 18% (19,21, 23,25-39), as reported in Germany (39). In Eastern countries, e.g., Japan, China and Taiwan, EBV-positive gastric cancer comprises 5.6 to 13.5% of cases (19,21,29-36). In Russia, Galetsky et al. (37) reported a frequency of 8.7%. In the US (including Hawaii) and Mexico, 8.15 to 16% of gastric carcinomas were EBV positive (25-28).

In Brazil, Hayashi et al. (40) found EBV infection in 4 of 80 (5.0%) gastric carcinomas of Brazilian patients, and in 29 of 510 (5.7%) gastric carcinomas of Japanese patients. Few EBV-positive gastric carcinomas (9/33 cases or 27.3%) were classified as LELC. This might explain the lower rate of EBV infection observed in these tumors. In a study published in 2001, Koriyama et al. (23) reported 4.7 and 11.2% of EBV-positive gastric carcinomas in Brazilians of Japanese origin (N = 148) or not (N = 150),

respectively. Interestingly, all these EBV-positive gastric carcinomas were classified as conventional carcinomas. In the present study, the frequency of EBV infection in gastric carcinomas was 11.32%, and most of them were classified as LELC (4/6, 66.7%).

Occurrence in elderly persons and male predominance are common in EBV-associated gastric carcinomas (23,25,27,29,31-37), and these findings were also observed in the present study. However, the site where EBV-positive gastric carcinoma more often arises is a controversial issue in the literature. Some studies have reported that the cardia and middle portion of the stomach are more frequently involved (29-31,33,37), whereas others have found that the antrum was the most typical site (23,28,32). In the present study, the antrum was the most common anatomic site involved by EBV-positive gastric carcinomas. Moderately to poorly differentiated conventional carcinomas and diffuse histology have also been associated with EBV-positive gastric cancers (23,27-29,31-33,36, 37), but the prognosis does not seem to be influenced by these characteristics (27,36). Unfortunately, no additional information is available regarding the follow-up of the patients with EBV-positive gastric carcinomas included in the present study.

EBV infection in gastric carcinomas in São Paulo State, Brazil, as well as their clinicopathologic characteristics, are similar to those reported for other countries. Additional studies are needed in order to obtain information regarding the biological behavior of EBV-positive gastric carcinomas in Brazilian patients.

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