



Epstein-Barr virus DNA associated with gastric adenocarcinoma and adjacent non-cancerous mucosa in patients from Manaus, Brazil

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ABSTRACT. Gastric cancer is one of most frequent causes of death in Brazil. The city of Manaus has one of the highest incidences of this disease in Brazil. The Epstein-Barr virus (EBV) is a ubiquitous herpesvirus that is classified as a group 1 carcinogen by the International Agency for Research on Cancer. We obtained biopsies from 6 control subjects and 10 patients with gastric carcinomas living in Manaus. In the patients, the samples were taken from tumors and from adjacent non-cancerous mucosa. These samples were screened for EBV DNA by PCR to amplify the 288-bp fragments from the Bam M region. The

EBV DNA was detected in 8 of the 10 tumor cases and in none of the 6 control subjects. In the positively identified samples, EBV DNA was detected in five corresponding resection margins. Previous research indicated only a weak association between EBV and gastric cancer. We suggest that EBV should be considered as a risk factor for gastric adenocarcinomas in Manaus.

Key words: Epstein-Barr; Gastric cancer; Amazon; Resection margin; Polymerase chain reaction

INTRODUCTION

Gastric cancer (GC) is one of the most common cancers, and is ranked 2nd among the worldwide cancer mortality rates (Ebert et al., 2006). Several factors may contribute individually or together to the emergence of this neoplasia, including infection with *Helicobacter pylori*, dietary habits, genetic factors, and viral infections (Schulz, 2005). GC is mostly found in individuals aged over 50 years; its incidence in individuals under 40 years corresponds to only 5% of all cases (Theuer et al., 1996; Mauad et al., 2000). Most patients are diagnosed with GC in an advanced state, thus drastically reducing the treatment options, survival, and cure (Ebert et al., 2006). In the northern part of Brazil, GC is the 2nd most common cancer in men and the 4th in women, accounting for an estimated 13 and 7 new cases per 100,000 per year for men and women, respectively (Guerra et al., 2005; INCA, 2012). While most GCs are correlated with *H. pylori* infection (Asaka et al., 1994; Komoto et al., 1998), such is not true for cases in the Brazilian State of Amazonas, where most GC patients are *H. pylori* negative. These facts motivated our group to investigate other risk factors to help explain this high incidence.

The incidence of the Epstein-Barr virus (EBV) with GC has been found to vary from 2 to 18% in the world population. A recent Brazilian report described an incidence of ~8% among GCs from Ceará (Lima et al., 2011). Thus, we considered investigating whether EBV could be correlated with GC in the Amazon.

EBV is classified as a group 1 carcinogen according to the International Agency for Research on Cancer (IARC). It belongs to the gamma-herpes family, which has a linear DNA molecule responsible for encoding approximately 100 proteins (Silva and Zucoloto, 2003; Lima and Rabenhorst, 2006). Reports describe more than 90% of our population as being previously exposed to EBV, which can be easily transmitted by salivary contact (Macswen and Crawford, 2003). This virus has 2 distinct life cycles in the human host: a lytic cycle, during which the production of new virions occurs, and a latent form, wherein it remains in a dormant state for the lifetime of the host (Pattle and Farrell, 2006). EBV is mostly well known as the causative agent for infectious mononucleosis, which is usually manifested in adulthood through latent EBV genes (Asaka et al., 1994). In addition to GC, EBV has been associated with malignancies including Burkitt's lymphoma, Hodgkin's disease, peripheral T-cell lymphoma, thymoma, and nasopharyngeal carcinoma (Lima and Rabenhorst, 2006). The role of EBV in tumorigenesis is not well understood, but there is evidence correlating its persistence in human epithelial cells with tumorigenesis (Tsao et al., 2012).

MATERIAL AND METHODS

Subjects

This study was approved by the Ethics Committee Review Board from the Federal University of the Amazon (CEP/UFAM: MEMO - No. 0057.0.115.000-11-CAAE). After signing informed consent, tumor specimens with resection margins were obtained after surgery from 10 GC patients, 4 females. Six biopsies were obtained from control subjects during upper endoscopy; 5 were from females. Subjects were classified as controls if no traces of cancer were detected by endoscopic evaluation. All biopsies were obtained from the stomach. Each biopsy was then subtyped and the clinical stage of the disease was determined according to the tumor, node, and metastasis classification (TNM) from the American Joint Committee on Cancer (AJCC).

DNA extraction

For the polymerase chain reaction (PCR) analysis, the biopsies were pulverized and genomic DNA was isolated by digestion in 500 μ L containing 10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 2% SDS, 10 mM EDTA, pH 8.0, and 15 μ L proteinase K (10 mg/mL). The material was incubated at 56°C for 2 h; DNA was then extracted by phenol-chloroform-isoamyl alcohol and resuspended in sterile distilled water. The extracted genomic DNA was submitted to PCR to confirm DNA integrity using p53 primers to exon 5, generating a 274-bp product (Pestaner et al., 1994).

PCR for detecting EBV DNA in the biopsies

The detection for EBV DNA used the primers TC67 and TC69, which amplify a portion of the Bam M region of EBV DNA and produce a 288-bp product (Saito et al., 1989). Amplification conditions consisted of an initial denaturation step at 95°C for 5 min followed by 35 cycles of 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min. This was followed by a final extension step at 72°C for 5 min. For all reactions we used positive (*viz.*, DNA from Raji cells) and negative (*viz.*, Milli-Q water) controls (Lattario et al., 2008).

Diagnosis of EBV DNA

Diagnosis was accomplished by visually inspecting for the presence of 288-bp PCR products using a 10% polyacrylamide gel stained with silver nitrate (Rosenbaum and Riesner, 1987). The approximate size of the amplified fragments was calculated using co-electrophoresis with low molecular weight (BioRad).

RESULTS

The results from our analysis for the presence of *H. pylori* and EBV DNA are presented in Table 1. Briefly, EBV DNA was detected in 80% of the biopsies, among which 50% had positive resection margins. EBV DNA was not found in any control subjects.

Table 1. Clinical and experimental features for the presence of Epstein-Barr virus (EBV) from subjects enrolled in the study.

No.	Type	Gender	Age (years)	Diagnosis	<i>Helicobacter pylori</i>	EBV DNA cancer	EBV DNA RM
1	Control	F	46	Gastritis	-	-	NA
2	Control	F	26	Gastritis	-	-	NA
3	Control	F	52	Gastritis	-	-	NA
4	Control	M	43	Gastritis	-	-	NA
5	Control	F	55	Gastritis	-	-	NA
6	Control	F	62	Gastritis	-	-	NA
7	Cancer	F	49	T4N3M0	-	+	+
8	Cancer	F	71	T3NXM0	-	+	+
9	Cancer	M	48	T4NXM0	-	-	-
10	Cancer	F	46	T4N0M0	-	-	-
11	Cancer	F	46	T4NXN0	+	-	+
12	Cancer	M	47	T3N3M0	+	+	-
13	Cancer	M	68	T3NXM0	-	+	+
14	Cancer	M	50	T4NXM1	-	+	+
15	Cancer	M	40	T1NXM0	-	-	+
16	Cancer	M	62	T4N2M1	-	+	+

RM = resection margin; F = female; M = male; (+) or (-) = presence or not of *H. pylori* and EBV, respectively; NA = not applicable.

DISCUSSION

Previous studies have described *H. pylori* as the main causative agent for GC (Asaka et al., 1994; Komoto et al., 1998) and report a low correlation between EBV and GC (Lima and Rabenhorst, 2006; Lima et al., 2011). Here, EBV DNA was detected in 80% of the tumors examined using PCR, and 50% presented EBV DNA in both the GC specimen and the resection margin. EBV was detected in both early and advanced stages of GC, as aligned with previous reports (Shibata et al., 1991; Sample and Sample, 2008). It is also important to emphasize that *H. pylori* was detected in only 2 cases. As such, although EBV's mechanisms for triggering carcinogenesis are not well understood (Nishikawa et al., 1999), our results suggest that EBV should be considered as a risk factor for this disease, especially in the Amazon State, Brazil.

An increased sample size will be necessary to accomplish a more robust statistics. Regardless, we have raised an important hypothesis of a high correlation between EBV and GC in the Amazon that deserves further investigation by the scientific community in different and larger cohorts.

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