aged older than 60 than among those aged 60 or younger (15). Koriyama et al. (7), examining by far the largest number of EBVaGCs in Japan, showed the age distributions of intestinal- and diffuse-type EBVaGCs to be different from each other: the estimated incidence of intestinal-type EBVaGCs had its peak in patients in their seventies, while diffuse-type EBVaGCs appeared to have a much older peak incidence, if any. Their results suggest that the natural history of EBVaGC may differ in those two histologic subtypes. However, the discrepancies of age dependence among various countries do not appear to be explained by differences in the histologic distributions in those countries. In the present study, we could not test the tendency presented by Koriyama et al. due to the small number of EBVaGCs in our study. To understand the sex and age distribution of EBVaGC, further studies on the prevalence of EBV infection, age at infection with EBV, and EBV genotypes seem warranted. It may also be of interest to compare lifestyles and genetic backgrounds such as human leucocyte antigen (HLA) in EBVaGCs and EBV-negative GCs, since HLA distribution was reported to be different between EBVaGCs and EBV-negative GCs (16).

EBVaGC is known to show uniform EBER staining in all the carcinoma cells (2). That was also the case in the present study except for Case 5 (Table II), where EBER-1 staining was observed in the carcinoma in the middle part of the stomach but not in the carcinoma in the antrum. We suspect that this is the case of double synchronous carcinomas, one in the stomach antrum (EBER-1 negative) and the other in the body of the stomach (EBER-1 positive). To our knowledge, this is the first report of possible synchronous double adenocarcinomas that could not be identified unless EBER-1 ISH was conducted. Recently, however, zur Hausen et al. reported that EBV can only infect neoplastic gastric cells, and thus the infection is a late event in gastric carcinogenesis (17). We cannot deny the other possibility that the observed double adenocarcinomas have arised from the same tumor with only one area infected by EBV.

In summary, the present study showed that the proportion of EBVaGCs in Peru was 3.9%. The figure is the lowest among those reported in Latin American countries. An interesting feature of Peruvian EBVaGC was a lack of a male predominance. Other clinicopathological features of EBVaGC in Peru were similar to those found in literature: EBVaGC showed no age dependence, a predominance in the non-antrum part of the stomach, and high frequencies in histological subtypes of tub2 and por1. Further studies are needed to identify the cause of the low frequency and lack of male predominance of EBVaGC in Peru.

Acknowledgements: This work was supported by Grants-in-Aid for Scientific Research on Priority Areas of the Ministry of Education, Culture, Sports, Science, and Technology of Japan (12218231).

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Received: July 17, 2004

Chihaya Koriyama, M.D.
Department of Epidemiology and Preventive Medicine, Kagoshima University Graduate School of Medical and Dental Sciences,
8-35-1 Sakuragaoka,
Kagoshima 890-8544, Japan
Tel.: +81-99-275-5296; Fax: +81-99-275-5299
E-mail: fiy@m.kufm.kagoshima-u.ac.jp

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

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World J Gastroenterol 2005;11(39):6096-6103
World Journal of Gastroenterology ISSN 1007-9327

• *GASTRIC CANCER* •

Epstein-Barr virus-associated gastric carcinoma: Evidence of age-dependence among a Mexican population

Roberto Herrera-Goepfert, Suminori Akiba, Chihaya Koriyama, Shan Ding, Edgardo Reyes, Tetsuhiko Itoh, Yoshie Minakami, Yoshito Eizuru

Roberto Herrera-Goepfert, Department of Pathology, Instituto Nacional de Cancerologia, Mexico City, Mexico
Suminori Akiba, Chihaya Koriyama, Shan Ding, Department of Epidemiology and Preventive Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan
Edgardo Reyes, Department of Pathology, Instituto Nacional de Ciencias Médicas y de la Nutrición "Salvador Zubirán", Mexico City, Mexico
Tetsuhiko Itoh, Department of Pathology, Kagoshima Institute of Preventive Medicine, Kagoshima, Japan
Yoshie Minakami, Yoshito Eizuru, Division of Oncogenic and Persistent Viruses, Center for Chronic Viral Diseases, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan
Supported by a Grant No. 12218231 from Grants-in-Aid for Scientific Research of the Ministry of Education, Science, Sports, and Culture of Japan
Correspondence to: Roberto Herrera-Goepfert, MD, Departamento de Patología Instituto Nacional de Cancerología, Av. San Fernando #22, Colonia Sección XVI, Tlalpan, México DF 14080, Mexico. rhgoepfert@yahoo.com.mx
Telephone: +52-55-5628-0466 Fax: +52-55-5573-4662
Received: 2005-04-13 Accepted: 2005-05-24

Abstract

AIM: To investigate features of Epstein-Barr virus (EBV)-associated gastric carcinoma (EBVaGC) among a Mexican population.

METHODS: Cases of primary gastric adenocarcinoma were retrieved from the files of the Departments of Pathology at the Instituto Nacional de Cancerología and the Instituto Nacional de la Nutrición in Mexico City. The anatomic site of the gastric neoplasia was identified, and carcinomas were histologically classified as intestinal and diffuse types and subclassified as proposed by the Japanese Research Society for Gastric Cancer. EBV-encoded small non-polyadenylated RNA-1 (EBER-1) *in situ* hybridization was conducted to determine the presence of EBV in neoplastic cells.

RESULTS: We studied 330 consecutive, non-selected, primary gastric carcinomas. Among these, there were 173 male and 157 female patients (male/female ratio 1.1/1). EBER-1 was detected in 24 (7.3%) cases (male/female ratio: 1.2/1). The mean age for the entire group was 58.1 years (range: 20-88 years), whereas the mean age for patients harboring EBER-1-positive gastric carcinomas was 65.3 years (range: 50-84 years). Age and histological type showed statistically significant differences, when EBER-1-positive and -negative gastric carcinomas were compared. EBER-1 was detected in hyperplastic- and dysplastic-gastric mucosa surrounding two EBER-1-negative carcinomas, respectively.

CONCLUSION: Among Latin-American countries, Mexico has the lowest frequency of EBVaGC. Indeed, the Mexican population >50 years of age was selectively affected. Ethnic variations are responsible for the epidemiologic behavior of EBVaGC among the worldwide population.

Key words: Epstein-Barr virus; Stomach; Lymphoepithelioma-like carcinoma; Gastric carcinoma; EBV-A; EBER-1; LMP-1

Herrera-Goepfert R, Akiba S, Koriyama C, Ding S, Reyes E, Itoh T, Minakami Y, Eizuru Y. Epstein-Barr virus-associated gastric carcinoma: Evidence of age-dependence among a Mexican population. *World J Gastroenterol* 2005; 11(39): 6096-6103
http://www.wjgnet.com/1007-9327/11/6096.asp

INTRODUCTION

Gastric cancer (GC) is the second leading cause among cancer deaths in the world[1] and is one of the most frequent malignant neoplasms in Mexico[2]. Although the etiology of gastric carcinoma is now accepted as multifactorial, infectious agents play a central role in the mechanism of neoplastic transformation. The bacterium *Helicobacter pylori* (*H pylori*) has been implicated in a high percentage of gastric adenocarcinomas[3], in intestinal- as well as diffuse-type adenocarcinomas, according to the Lauren histoepidemiologic classification[4]. Another infectious agent, Epstein-Barr virus (EBV) or gamma type 4 herpes virus, has also been proved to be associated with gastric carcinoma in approximately 10% of cases[5]. This association has been reported in intestinal- and diffuse-type adenocarcinomas, as well as in nearly 100% of cases labeled lymphoid stroma-rich, lymphoepithelioma-like (LEL) carcinomas. The etiological role of EBV in GC development has been suspected on the basis of the uniform expression of Epstein-Barr nuclear antigen (EBNA)-1 protein, and EBV-encoded small non-polyadenylated RNA (EBER)-1 in all GC cells, the episomal monoclonality of the EBV genome, the elevated serum antibodies against EBV-related antigens among EBV-GC patients, and the unique 'lace pattern' morphology in some

early-stage EBV-GCs.

EBV-associated gastric carcinoma (EBVaGC) accounts for 1.7-16% of gastric carcinomas throughout the world, excluding LEL carcinomas[6]. The lowest frequency has been recorded in the UK, whereas the highest was in the USA. The definitive explanation for this figure remains unclear, but is probably related with genetic variations among different populations, as well as cultural and environmental influences among different geographic regions. Among Latin Americans, Mexican individuals are less likely to develop GC in association with EBV infection; in a previous study, we reported a prevalence of 8.15%[7]. In these series, diffuse-type EBV-GCs were seen exclusively, and EBER-1 was demonstrated in 100% of LEL carcinomas. In the present study, we expanded the number of cases under scrutiny and provided evidence that the risk for EBVaGC was significantly increased among patients >50 years of age in Mexico.

MATERIALS AND METHODS

Patient population

We retrieved cases of gastric adenocarcinoma from the files of the Departments of Pathology at the Instituto Nacional de Cancerología (1983-2000) and the Instituto Nacional de la Nutrición (1980-1995) in Mexico City. The results of a partial analysis of 135 cases were published previously elsewhere[7]. Eligible cases were included whenever they possessed complete demographic and pathologic information, as well as paraffin blocks with appropriate and well-preserved neoplastic tissue for molecular analysis. The age and gender of patients, and anatomic site, histological type, and depth of invasion of gastric carcinomas were obtained from records at the corresponding Department of Pathology.

Pathologic features

The anatomic site of gastric neoplasia was identified as upper (proximal) third, middle third, or lower (distal) third[8]. On the basis of predominant histological pattern, carcinomas were classified as intestinal- or diffuse-type according to the Lauren criteria[4] and subclassified as proposed by the Japanese Research Society for Gastric Cancer as follows[9]: intestinal types tub1 (well-differentiated adenocarcinoma with distinct glandular pattern and columnar epithelium throughout, moderate or small amount of stroma); tub2 (moderately differentiated adenocarcinoma with small or incomplete tubular structures with cubical or flat epithelium, amount of stroma variable from case to case), and muc (mucinous carcinoma); diffuse types, including por1 (poorly differentiated adenocarcinoma with solid, sheet-like proliferation with an alveolar pattern and indistinct tubular differentiation), por2 (poorly differentiated adenocarcinoma with acinar and trabecular pattern, usually showing diffuse infiltration with abundant fibrous stroma), and sig (signet-ring cell carcinoma). A special category, LEL carcinoma, similar to por1 adenocarcinoma but with dense lymphoid infiltrate exceeding total mass of carcinoma cells, was included. The depth of invasion was specified as mucosa; submucosa; or muscularis propria, subserosa, or serosa.

In situ hybridization

Molecular analysis was conducted as previously described[10]. Briefly, we retrieved one representative formalin-fixed, paraffin-embedded tissue sample from each carcinoma containing the neighboring non-neoplastic gastric mucosa. Two slides with 5 μm sections were prepared from each paraffin block. A set of slides were conventionally stained with hematoxylin and eosin, whereas the remainder were enhanced for EBER-1 *in situ* hybridization. The remaining paraffin-block sections were deparaffinized, rehydrated, predigested with pronase, prehybridized, and hybridized overnight at 37 ℃ with a concentration of 0.5 ng of digoxigenin-labeled probe. After sections were washed with 0.5×saline sodium citrate, hybridization was detected by anti-digoxigenin, antibody-alkaline phosphatase conjugate. Sections from a patient with known EBV-positive gastric carcinoma were used for a positive control, and sense probe to EBER-1 was used for a negative control for each procedure.

EBV genotyping

Preparation of DNA Each formalin-fixed and paraffin-embedded specimen was cut into 10-μm-thick slices, and a DNA sample was prepared following the method reported previously[11]. Each deparaffinized sample was treated with proteinase K (200 μg/mL) at 37 ℃ overnight followed by phenol/chloroform extraction and ethanol precipitation. Finally, the extracted DNA sample was dissolved in 50 μL of TE buffer.

Genotype-specific primer sets and probes Four different regions, the EBNA-3C, *Bam*HI-F, *Bam*HI-I, and *Xho*I sites in LMP-1, were used to determine viral genotypes. Types A and B can be determined by using the EBNA-2, -3A, -3B, or -3C gene[12-14]. In the present study, we chose EBNA-3C for genotyping because we experienced a higher detection rate of the primer set than those of the EBNA-2 region found in previous studies[15,16]. Types A and B, identified by PCR amplification of EBNA-3C region, corresponded to a 153- and a 246-bp band, respectively, and were confirmed by Southern blot hybridization with type-specific internal probes [14]. Wild-type F and f variant were identified by the presence of a 186-bp fragment in amplification of the *Bam*HI F region; after *Bam*HI cleavage, a 186-bp fragment could be identified in the case of wild-type F, and a 127-bp fragment could be identified in the case of the f variant. Wild-type F and f variants were confirmed by Southern blot hybridization with the internal probe as described previously[15].

For the *Bam*HI-I region, a 205-bp fragment was amplified by using primer sets as described previously[17], and types C and D were distinguished after cleavage by *Bam*HI-restriction enzyme. Type C had a 205-bp fragment, and type D had cleaved fragments with 130 and 75 bp. Types C and D were also confirmed by Southern blot hybridization with a cloned *Bam*HI-I DNA fragment probe.

To detect the *Xho*I polymorphism in exon 1 of the LMP-1 gene, we amplified a 497-bp DNA fragment with a primer set as previously described[18]. When two fragments, 340- and 157-bp long, were observed after *Xho*I digestion of the PCR product, the case was considered to contain the *Xho*I cleavage site. The 497-bp fragment of the PCR product of the B95-8 cell line was used as a probe to confirm the *Xho*I

cleavage site of LMP-1 by Southern blot hybridization[19].

PCR and Southern blot hybridization The PCR template contained the appropriate primer pair (1 μmol/L each), deoxyribonucleotide triphosphates (200 μmol/L each), and *Taq* polymerase (Takara Shuzo, Kyoto, Japan) in a total of 100 μL of PCR buffer. PCR products or PCR products digested with *Bam*HI and *Xho*I were confirmed by electrophoresis in 2% agarose gel and by staining with 0.5 μg/mL of ethidium bromide. Then, electrophoretic pattern was photographed under ultraviolet light. Electrophoretic DNA was transferred onto a Hybond N^+ nylon membrane (Amersham Pharmacia Biotech, UK) by capillary blotting using 0.4 N NaOH solution. Membranes were prehybridized with hybridization buffer for 0.5-1 h at 42 ℃. After the probe was added, hybridization was carried out overnight at 42 ℃. Probes of types A and B, and *Bam*HI-F were labeled with Dig oligonucleotide 3'-end labeling kit and detected using a Dig luminescent detection kit (Boehringer Mannheim, Germany). For detecting the *Bam*HI-I fragment and *Xho*I polymorphism in LMP-1, hybridization was carried out using the ECL direct labeling and detection kit (Amersham Pharmacia Biotech, UK) according to the manufacturer's instructions.

Statistical analysis

Odds ratios (ORs) and 95% confidence intervals (95%CIs) were obtained from logistic regression analysis, making comparisons between EBER-1-positive and EBER-1-negative gastric carcinomas with regard to age, gender, decade, anatomic site, histologic type, and depth of invasion.

RESULTS

Patient characteristics

We studied 330 consecutive, non-selected cases of gastrectomies due to primary gastric carcinoma. Among the 330 cases, there were 173 male and 157 female patients. The mean age was 58.1 years (range: 20-88 years) for all the patients, 59.9 years (range: 22-88 years) for male patients, and 56.1 years (range 20-88 years) for female patients. EBER-1 was detected in 24 (7.3%) of the 330 cases, 13 in men (7.5%) and 11 in women (7.0%). The mean age for patients harboring EBER-1-positive gastric carcinomas was 65.3 years: male patients 66.2 years (range: 51-74 years) and female patients 64.4 years (range: 50-84 years). The male/female ratio was 1.1/1 for the entire group and 1.2/1 for those with EBER-1-positive carcinomas.

Pathologic findings

With regard to the anatomic site of the primary neoplasia, 44 (13.3%) carcinomas were localized in the upper-third, 128 (38.8%) were in the middle portion, and 156 (47.3%) were in the lower-third of the stomach. In two cases (one male and one female), the anatomic location could not be determined; the entire stomach showed neoplastic infiltration in the male patient, and information on the original location of primary neoplasia was not available in the female patient. Both cases were EBER-1-negative. The distribution of carcinomas according to anatomic site and histological type, and the anatomic site and histological type of EBER-1-positive carcinomas are shown in Tables 1 and 2, respectively. Fourteen cases corresponded to early carcinomas, and only 4 were confined to mucosa; 10 cases invaded the submucosal layer. The remaining 316 cases were advanced carcinomas affecting muscular, subserosal, and serosal layers, as well as adjacent organs. EBER-1 was positive in all LEL carcinomas, in 4 out of 141 intestinal-type adenocarcinomas and in 11 out of 180 diffuse-type adenocarcinomas. The EBER-1 *in situ* hybridization signal was uniformly distributed in the nuclei of all 24 positive cases (Figures 1-6). A characteristic

Table 1 Distribution of EBER-1-positive gastric carcinomas by anatomic site[1] and gender

	Total (EBER-1+/total) %		Males (EBER-1+/total) %		Females (EBER-1+/total) %	
Total	24/330	7.3	13/173	7.5	11/157	7.0
Upper	3/44	6.8	3/31	9.7	0/13	0
Middle	13/128	10.2	7/67	10.4	6/61	9.8
Lower	8/156	5.1	3/74	4.1	5/82	6.1

[1]In two cases, anatomic location could not be determined. All (one male and one female) were EBER-1-negative.

Table 2 Distribution of EBER-1-positive gastric carcinomas by histologic type and gender

	Total (EBER-1+/total) %		Males (EBER-1+/total) %		Females (EBER-1+/total) %	
I-type	4/141	2.8	3/87	3.5	1/54	1.9
Tub1	0/42	0	0/28	0	0/14	0
Tub2	4/80	5.0	3/47	6.4	1/33	3.0
Muc	0/19	0	0/12	0	0/7	0
D-type	20/189	10.6	10/86	11.6	10/103	9.7
Por1	8/64	12.5	3/31	9.7	5/33	15.2
Por2	2/45	4.4	2/19	10.5	0/26	0
Sig	1/71	1.4	1/32	3.1	0/39	0
LEL	9/9	100	4/4	100	5/5	100

I-type: Intestinal-type adenocarcinoma; *D-type*: Diffuse-type adenocarcinoma.

lace pattern was evident in the intramucosal component of three EBER-1-positive carcinomas, two por1 plus tub2 and one tub2 plus por1 adenocarcinomas. Twenty-two of twenty-four EBER-1-positive cases extended beyond the submucosa, whereas two carcinomas, one from a female and one from a male patient, did not exceed the submucosal layer.

There were two EBER-1-negative carcinomas accompanied by EBER-1-positive gastric lesions. The first case, a 52-year-old male patient (Figures 7 and 8), had EBER-1 expression in regenerative epithelium of gastric mucosa adjacent to an EBER-1-negative primary adenocarcinoma (por1). The second case was a 46-year-old female patient whose EBER-1-negative adenocarcinoma (por1) was in the immediate vicinity of dysplastic gastric glands with EBER-1 expression (Figures 9 and 10).

Among the demographic and pathologic variables analyzed, age and histologic type had statistically significant differences, when EBER-1-positive and EBER-1 negative gastric carcinomas were compared (Table 3). In addition, comparison among patients more or less than 60 years of age showed significant differences ($P = 0.008$).

EBV genotype

We examined the genotype of seven EBV strains detected from

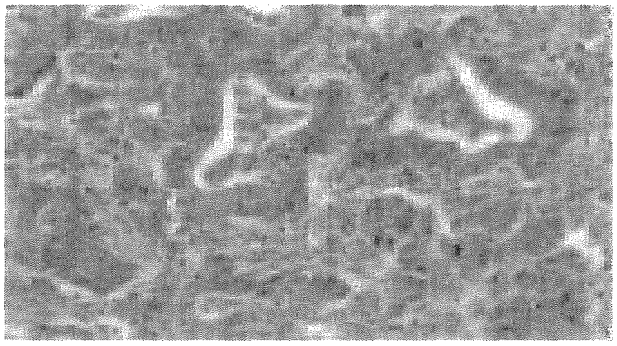

Figure 1 Moderately differentiated, intestinal-type (tub2) adenocarcinoma. Irregular neoplastic tubular structures are seen throughout the field (hematoxylin and eosin stain).

Figure 4 Same case as in Figure 3. A uniform nuclear signal of EBER-1 is seen in neoplastic cells (*in situ* hybridization).

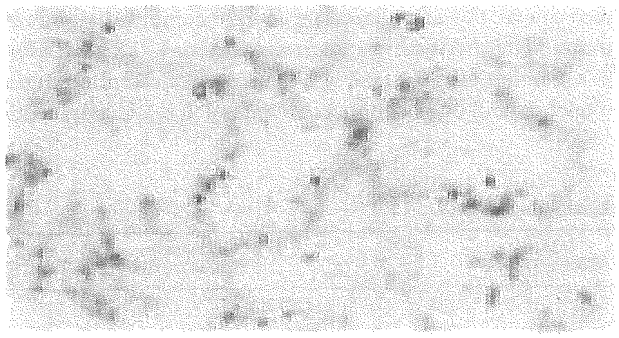

Figure 2 Same case as in Figure 1. EBER-1 nuclear positivity is limited to neoplastic cells lining the tubular structures (*in situ* hybridization).

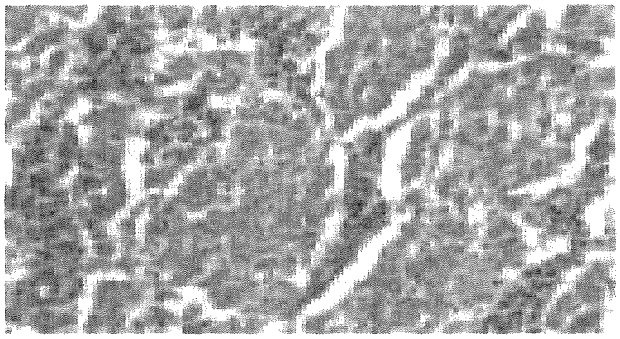

Figure 5 Poorly differentiated, LEL carcinoma. Clusters of neoplastic cells are separated by lymphoplasmacytic infiltrate.

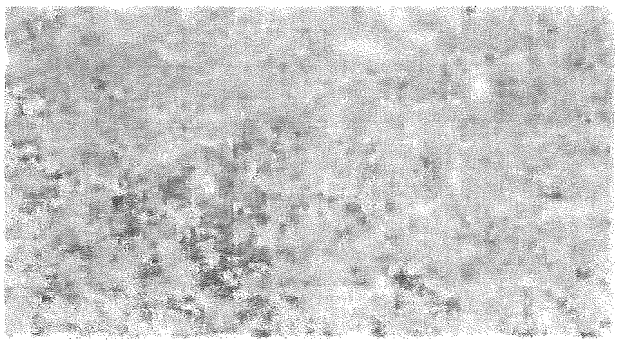

Figure 3 Diffuse-type (por1) adenocarcinoma. Sheets of neoplastic cells are distributed in an indistinct pattern (hematoxylin and eosin stain).

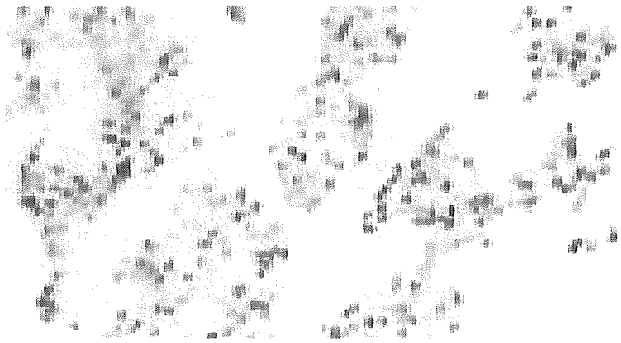

Figure 6 Same case as in Figure 5. An EBER-1-positive signal is detected in the nuclei of neoplastic cells (*in situ* hybridization).

EBER-1-positive cases; genotype could be determined in five of them. All were type A, wild-type F, and type D. In analysis of the *Xho*I cleavage site in LMP-1, we found that the cleavage site was lost in four cases and was maintained in one case.

Figure 7 Lining gastric epithelium shows regenerative changes characterized by nuclear growth without atypia. There are few neoplastic cells in the underlying lamina propria (hematoxylin and eosin stain).

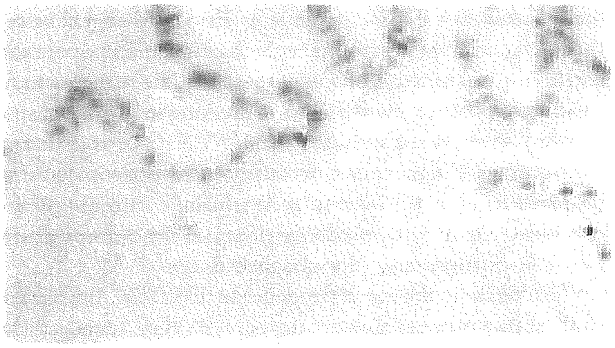

Figure 8 Same case as in Figure 7. The EBER-1-positive nuclear signal is restricted to regenerative epithelium. Note the EBER-1 nuclear negativity of neoplastic cells infiltrating the lamina propria (*in situ* hybridization).

Figure 9 Lining gastric epithelium shows high-grade dysplasia, characterized by cell stratification and crowding, increased nuclear/cytoplasm ratio, nuclear atypia, and prominent eosinophilic nucleoli (hematoxylin and eosin stain).

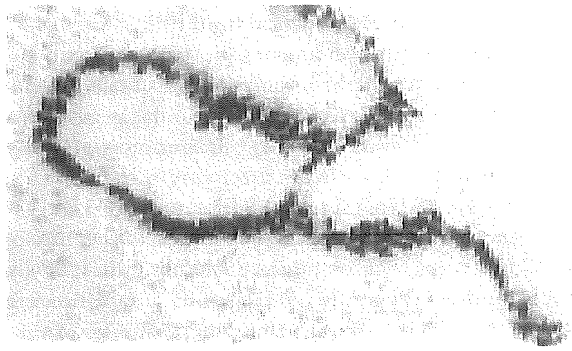

Figure 10 Same case as in Figure 9. Dysplastic epithelium is intensely positive for the EBER-1 nuclear signal (*in situ* hybridization).

Table 3 Comparison of demographic and pathologic variables between EBER-1-positive and EBER-1-negative gastric carcinomas[1]

	EBER-1+/total	OR	95%CI	*P*
Gender				0.859
Female	11/157	1	Reference	
Male	13/173	0.9	0.4-2.2	
Age (yr)				0.013
20-49	0/87	<0.1		
50-69	14/170	0.6	0.2-1.3	
70-88	10/73	1	Reference	
Decade				0.787
1980-1989	11/130	1	Reference	
1990-2000	13/200	0.9	0.4-2.1	
Tumor site				0.229
Cardia	3/35	1.9	0.5-7.5	
Middle	13/137	2.2	0.9-5.5	
Antrum	8/156	1	Reference	
Lauren classification				0.005
Intestinal	4/141	1	Reference	
Diffuse	20/189	4.9	1.6-14.8	
Depth				0.273
Early	2/14	2.4	0.5-11.8	
Advanced	22/316	1	Reference	

[1]Odds ratios and 95% confidence intervals were obtained from logistic analysis. Age was adjusted in the analysis of variables other than age.

DISCUSSION

In this study, we found a 7.3% prevalence of EBVaGC in Mexico. In Latin America, this frequency is in contrast with that reported by Koriyama *et al.*, (11.2%)[20] and Lopes *et al.*, (11.3%)[21] in Brazil, Carrascal *et al.*, in Colombia (13%)[22], and Corvalan *et al.*, in Chile (16.8%)[23]. Excluding LEL carcinomas, the prevalence of EBVaGC in Mexico was 4.7%, whereas in Chile it was 15.8%. In a Brazilian study by Koriyama *et al.*[20], and a Colombian study by Carrascal *et al.*[22], there were no LEL carcinomas. Nonetheless, in the study by Lopes *et al.*[21], a high prevalence of LEL carcinomas (66.7%) among EBVaGC patients was found in a Brazilian population; thus, the prevalence of EBVaGC excluding LEL carcinomas is the lowest (3.8%). Conversely, the prevalence of LEL carcinoma was 7.6% in Brazil, 2.7% in Mexico, and 1.1% in Chile. The male/female ratio (1.2/1) was, as previously noted[7], the lowest among the series reported worldwide. Moreover, after excluding LEL carcinomas, Mexico remains among countries with the low prevalence of EBVaGC worldwide[6].

The frequency of EBVaGC among GC patients of Mexican ancestry in the USA ranged from 10.2%[24] to 12%[25], which is higher than the frequency (7.3%) reported by us. This peculiar migratory phenomenon has also been seen in other countries such as Japan and China. In Japan, the mean frequency of EBVaGC is 6.2%, but among patients of Japanese descent, those who are living in Hawaii, the frequency is 10.2%. In Taiwan, the frequency of EBVaGC among patients of Chinese descent is 11.2%, in comparison to 6.8% in China[26]. This figure probably indicates that besides ethnic and genetic backgrounds, environmental factors are involved in the development of EBVaGC.

A high frequency of EBVaGC at older ages is evident in our Mexican study. Not a single case of EBVaGC was observed among patients aged <50 years. This feature was previously highlighted by Gulley *et al.*[25], who examined American patients of Mexican descent in the USA and found EBVaGC cases only among those aged 56 years or older. Age dependence of EBVaGC frequency was statistically significant in their study ($P = 0.04$). The absence of EBVaGC in a set of patients of Mexican ancestry aged <56 years was also reported by Vo *et al.*[24], although the age difference they reported was not statistically significant. A similar age dependence was reported in China[26], where EBVaGC frequency was higher among those aged 60 years or older than those aged <60 years ($P = 0.03$); interestingly, the frequency of EBVaGC (7.8%) in their study is quite similar to that reported by us (7.3%).

In Brazil, Lopes *et al.*[21], also did not find any patient less than 52 years of age, although other Latin-American studies such as those of Koriyama *et al.*[20], and Corvalan *et al.*[23], did not show any age dependence, reporting EBVaGC cases in patients <50 years. Contrary to the age dependence observed in the present study, a large-scale Japanese study reported a high prevalence of EBVaGC in young men[27]. Furthermore, the same authors showed a significant decreasing trend in EBV prevalence with increasing age for males ($P = 0.04$). Carrascal *et al.*[22], also reported an age-dependent decrease of EBVaGC frequency among Colombian individuals with GC (P for trend = 0.022).

The fact that EBV-associated cancer cannot be detected in other digestive tract organs including the colon and esophagus indicates the importance of epithelial change(s) specific to the stomach[28]. EBV-latent infection products were reported to be expressed in predisposing conditions for gastric carcinoma[29,30]. Our observation showing that EBVaGC could not be found among patients <50 years of age supports the involvement of gastric-mucosal changes occurring late in human life in Mexico, as well as in Brazil and China, and relatively early in Japan and Colombia.

EBVaGC has been related to atrophic gastritis, and EBV DNA has been isolated from epithelial cells in gastric mucosa carrying chronic atrophic gastritis[29-31]. Indeed, intestinal metaplasia may enhance EBV entrance into epithelial cells via adherence of the virus to the secretory component of polymeric immunoglobulin A[32]. Our finding of two cases of EBV non-associated gastric carcinoma, one positive for EBER-1 in adjacent hyperplastic mucosa -a finding not previously described -and the other with an EBER-1-positive signal in dysplastic mucosa -a finding originally reported by Shibata and Weiss[33] -also suggests that the most plausible mechanisms for EBV entry into gastric epithelial cells are those related to previous mucosal damage and cooperation with some unknown promoter factors. In the present study, we did not observe any EBER-1 expression in normal gastric mucosa, even surrounding LEL-EBVaGC or infiltrating lymphocytes. Furthermore, we analyzed endoscopic gastric biopsies from 116 Mexican individuals >40 years of age carrying gastritis with mild atypia, and we did not find any EBER-1-positive case (unpublished data).

In addition to the age dependence of EBVaGC, the present study shows other characteristics of EBVaGC such as distal presentation among female patients and no male preponderance, altogether supporting that ethnicity and genetic backgrounds may address this particular outcome of EBV infection in the Mexican population. Among genetic backgrounds, an immunogenetic constitution may influence the outcome of EBV infection. Human leukocyte antigens (HLA) of the major histocompatibility complex have been implicated in susceptibility to develop EBV-related malignancies[34]. Very recently, we reported an association between the *HLA-DQB1**0501 allele and GC, predominantly in those labeled as diffuse-type carcinomas[35]; unfortunately, EBV status could not be assessed.

In Mexico, EBV antibody prevalence at 4-6 years of age is about 75%[36]. All EBV strains detected in EBVaGC and subjected to EBV genotyping were type A. Previous molecular studies on nasal T-lymphocyte/natural killer-cell lymphomas (nT/NKL) in Mexico[37] documented that EBV type A (EBV-1) is more frequent than EBV type B (EBV-2), as in nT/NKL and sino-nasal-B-cell lymphomas, and as in reactive tonsils from healthy individuals, thus suggesting that viral infection with EBV-1 strain is highly predominant among the Mexican population. In addition, the same authors[37] found a similar incidence of EBV LMP-1 deletions in Mexican individuals harboring nT/NKL as compared with normal subjects. Mori *et al.*[38], found no significant differences in DNA sequences of the LMP-1 region of EBV strains isolated from EBVaGC patients and throat washing samples of healthy individuals. So far no studies

have revealed differences in the genotype of EBV detected in EBVaGC *vs* that found in healthy individuals.

In conclusion, EBVaGC occurs less in Mexico than among other Latin-American populations, but it is as frequent in male as it is in female patients >50 years. In Mexican women, EBVaGC affects the middle and distal portions of the stomach but not the proximal portion. Finally, the participation of sequential steps in the mechanism of neoplastic transformation in EBVaGC, in a similar manner to the cascade of events described by Correa[39] in gastric carcinogenesis, cannot be ruled out.

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Science Editor Guo SY **Language Editor** Elsevier HK

Rev Méd Chile 2005; 133: 753-760

ARTÍCULOS DE INVESTIGACIÓN

Características clínico-moleculares del cáncer gástrico cardial asociado al virus Epstein Barr

Alejandro Corvalán R[1,2], Suminori Akiba[3], María Teresa Valenzuela B[4], Miguel Angel Cumsille G[4,a], Chihaya Koriyama[3], Jorge Argandoña C[2,b], Claudia Backhouse E[2,c], Matilde Bal C[4], Fernando Mena U[5], Mariana Palma V[2,c], Yoshito Eizuru[3].

Clinical and molecular features of cardial gastric cancer associated to Epstein Barr virus

***Background**: Mortality caused by cardial gastric cancer in Chile, is increasing. Previously we demonstrated an association between Epstein Barr virus and this specific location of gastric cancer. **Aim**: To perform a clinical and molecular characterization of cardial gastric cancer associated to Epstein Barr virus. **Material and methods**: Epstein Barr virus was identified in 93 cardial gastric tumors, by in situ hybridization. Clinical and pathological features, survival and expression of p53 and c-erbB2 were compared between tumors with or without the presence of the virus. **Results**: Twenty two (23.6%) tumors expressed Epstein Barr virus. No difference in sex or age of patients with tumors positive or negative for the virus was observed. Epstein Barr positive tumors had a tendency to have a higher frequency of Bormann III endoscopic appearance and a lower frequency of p53 accumulation (p=0.06). Five years survival was 67% and 42% of tumors positive and negative for the presence of the virus, respectively (p=0.57). **Conclusions**: Our results, although not significant, show a tendency towards unique characteristics of cardial gastric tumors associated to Epstein Barr virus (Rev Méd Chile 2005; 133: 753-60).*

*(**Key Words**: Epstein-Barr virus infections; Herpes virus 4, human; Stomach neoplasms)*

Recibido el 1 de septiembre, 2004. Aceptado en versión corregida el 30 de marzo, 2005.

Trabajo financiado por *Grants in Aid for Scientific Research* (12218231) e *International Scientific Research, Special Cancer Research* (09042007) Ministerio de Educación, Japón y Dirección Académica, Clínica Las Condes, Chile (Proyecto Her2/neu y Cáncer Gástrico de ubicación cardial).

[1]Departamento de Anatomía Patológica, Pontificia Universidad Católica de Chile. [2]Instituto Chileno Japonés de Enfermedades Digestivas, Hospital Clínico San Borja Arriarán, Santiago, Chile. [3]Departamento de Salud Pública y Centro de Enfermedades Virales Crónicas, Facultad de Medicina, Universidad de Kagoshima, Kagoshima, Japón. [4]Escuela de Salud Pública, Facultad de Medicina, Universidad de Chile, Santiago Chile. [5]Departamento de Patología, Hospital Max Peralta, Cartago, Costa Rica.

[a]Magíster en Bioestadística

[b]Licenciado en Tecnología Médica

[c]Tecnóloga Médica

Correspondencia a: Dr. Alejandro Corvalán R. Laboratorio Biología Molecular, Departamento de Anatomía Patológica, Facultad de Medicina, Pontificia Universidad Católica de Chile. Lira 85, Santiago. Casilla 114-D. Teléfono: 56(2) 3543209. Fax: 56(2) 6395101. E-mail: corvalan@med.puc.cl

El cáncer gástrico (CG) representa la primera causa de muerte por enfermedades neoplásicas en Chile y aunque su tasa de mortalidad se ha estabilizado[1], la frecuencia del CG de ubicación cardial se encuentra en aumento[2-4]. En efecto, autores chilenos[2,3] han demostrado que los tumores cardiales han incrementado entre 12 y 15% en los últimos 30 años, llegando a representar el 42,3% de las localizaciones. De modo similar, investigadores americanos[4] han reportado un incremento anual de 4,4% entre 1976 y 1987, sin observar modificaciones en la frecuencia del CG, en otras localizaciones. Se ha postulado que el CG cardial, sería una forma particular de CG, dado que se comunica una peor sobrevida a 5 años en esta localización en comparación con CG de otras ubicaciones[5]. Por otra parte, se ha descrito que la frecuencia de mutaciones de los oncogenes p53 y Her2/neu sería diferente entre CG cardial y antral[6,7] y Hansen et al[8] han señalado que *Helicobacter pylori* no tendría un rol tan relevante en la patogénesis del CG cardial como el observado en la ubicación antral. Incluso se ha observado un probable rol protector de *Helicobacter pylori* en el desarrollo del CG cardial[9]. Tomadas en conjunto, estas observaciones sugieren que el CG cardial sería una forma emergente y particular de CG.

El virus de Epstein-Barr (VEB), tradicionalmente asociado a neoplasias linfoides[10], también ha sido descrito en tumores epiteliales como carcinoma nasofaríngeo y linfoepiteliomas de distintos órganos, incluyendo el estómago[11,12]. En esta ubicación, los linfoepiteliomas no representan más de 5% de los tumores gástricos[13], sin embargo, en los últimos años se ha demostrado una emergente asociación entre VEB y CG[14]. En efecto, diversas publicaciones señalan una asociación de 6,9% a 18% entre VEB y CG[15-17] con características clínico-patológicas únicas, como predominio en hombres, localización alta (cardias y tercio medio del estómago) y una frecuencia similar de los subtipos "intestinal" y "difuso"[18]. Recientemente hemos analizado las características clínico-patológicas del CG asociado a VEB en Chile, encontrando una asociación de 16,8% (31/185), una de las más altas del mundo, y un perfil clínico-patológico único[19]. Este perfil se caracteriza por una distribución similar entre ambos sexos, una fuerte asociación con ubicación cardial y un predominio del patrón histológico "difuso"[19]. Dado que estas características han sido descritas en México[20] y en descendientes mexicanos en Estados Unidos[21], hemos sugerido la presencia de un perfil único del CG asociado a VEB en Latinoamérica[19].

Ya que el CG cardial representa una entidad propia y emergente en Chile y que una de las principales características del CG asociado a VEB es la fuerte localización cardial, el objetivo de nuestro estudio fue analizar características clínico-patológicas, moleculares y de sobrevida del CG cardial en sus formas asociada y no asociada al VEB.

Materiales y métodos

Pacientes. El estudio se realizó sobre la base de 305 piezas quirúrgicas de pacientes operados por CG en el Servicio de Cirugía del Hospital San Borja Arriarán y archivados en el Instituto Chileno-Japonés de Enfermedades Digestivas del mismo hospital entre 1993 y 1999. De este material y utilizando la definición de Locke et al[22] que definen como mucosa gástrica cardial a la región entre la unión gastroesofágica y los primeros 5 cm de estómago, se identificaron 93 casos, que son la base del presente estudio. Las características clínicas de estos casos se obtuvieron de la revisión de fichas médicas y las características patológicas de informes anátomo-patológicos correspondientes. Se consignó edad, sexo, tamaño tumoral (<5 y >5 cm), infiltración de pared gástrica y compromiso ganglionar (sin consignar número de ganglios comprometidos) de acuerdo a la Unión Internacional contra el Cáncer[23] y tipo histológico de acuerdo a la clasificación de Lauren[24]. En los tumores que infiltraban muscular propia o serosa, se consignó además la forma macroscópica según la clasificación de Bormann[25].

Hibridación in situ para la identificación del virus de Epstein-Barr. La presencia de VEB se realizó determinando la expresión del ARN pequeño no poliadenilado intranuclear-1 (EBER-1),

el producto viral más abundante de la infección latente de VEB[26]. La determinación se realizó con la sonda TTGCTAGGGAGGAGACGTGT complementaria a los nucleótidos 6653-6672 del gen EBER-1 de acuerdo al protocolo descrito por Chang et al[27].

Inmunohistoquímica para la identificación de p53 y Her2/neu. La determinación de p53 se realizó por inmunohistoquímica utilizando el anticuerpo DO-7 (Dako) de acuerdo a protocolos establecidos[28]. Brevemente, se realizaron cortes de 4 µm que fueron montados en portaobjetos seguidos de desparafinación, rehidratación y digestión con Tripsina al 0,37%. A continuación, las muestras fueron incubadas con el anticuerpo DO-7 (1:10) a 4°C por 12 h seguidas de anticuerpo secundario conjugado con biotina (1:300) por 30 min a 37°C y complejo estreptavidina-peroxidasa (1:300) por 30 min a 37°C. Finalmente, las láminas fueron incubadas con diaminobenzidina (DAB) por 10 min a temperatura ambiente en presencia de H_2O_2 al 0,3% y contrateñidas con hematoxilina. La determinación de Her-2/neu se realizó con el anticuerpo policlonal pAB-1 (Dako) en condiciones similares a las descritas y considerando reacción positiva a la tinción específica en la membrana citoplasmática de células tumorales[29].

Estadística. Los resultados y las asociaciones con variables clínico-patológicas se estudiaron usando el método de chi-cuadrado (x^2) y los análisis de sobrevida usando el método de Kaplan-Meier. Estos análisis estadísticos se hicieron en el paquete computacional Stata 6.0.

Resultados

Hibridación in situ. La hibridación *in situ* se realizó en 93 casos, resultando 22 (23,6%) con tinción positiva. En los casos considerados con tinción positiva se observó la expresión de EBER-1 en forma uniforme y exclusiva en todos los núcleos de las células tumorales y no se observó expresión de EBER-1 en células epiteliales no tumorales ni linfocitos peritumorales (Figura 1).

Características clínicas y patológicas. Los resultados de la comparación de variables clínicas y anátomo-patológicas entre casos de CG cardial VEB+ y VEB- se muestran en la Tabla 1. Se observa que con relación al sexo y edad no hay diferencias significativas entre ambos grupos. Sin embargo, la edad más frecuente en que ocurrió la enfermedad, o modo, fue menor en el subtipo VEB+ (52 años vs 67 años). Con relación a variables anátomo-patológicas se observa que no hay diferencias en el tamaño tumoral como tampoco la infiltración de la pared gástrica entre los tumores VEB+ y VEB-. Entre los tumores T2-T4 (avanzados) observamos que aunque la forma Bormann III fue la más frecuente para ambos grupos, esta presentación macroscópica fue más frecuente en los tumores EBV+ (p=0,06). Con relación a la presentación histológica y basándose en la clasificación de Lauren, no se observaron diferencias entre los tipos "intestinal" y "difuso". En esta serie observamos 4 casos con abundante infiltrado linfocitario, los que fueron clasificados como linfoepitelioma gástrico y todos ellos correspondieron al grupo VEB+.

Características moleculares. La determinación de p53 se realizó en 21 casos VEB+ y en 57 casos VEB-. Se consideró tinción positiva con 10% o más

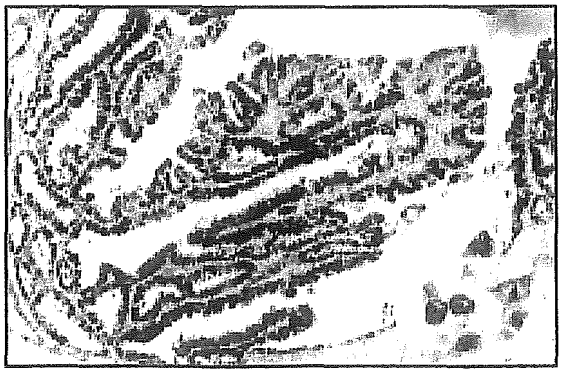

Figura 1. Hibridación *in situ* para EBER-1. Se observa expresión uniforme y exclusiva de EBER-1 en todos los núcleos de células tumorales de un cáncer gástrico cardial de tipo intestinal bien diferenciado (papilo-tubular), indicando la presencia de VEB (40X). Inserto: detalle con mayor aumento de la tinción nuclear.

Tabla 1. Correlaciones clínico-patológicas del cáncer gástrico de ubicación cardial asociado al virus de Epstein-Barr

	VEB positivo 22 casos		VEB negativo 71 casos		
	n	(%)	n	(%)	p
Sexo					
hombre	6	(27,3)	25	(35,2)	
mujer	16	(72,7)	46	(64,8)	0,49
Edad					
promedio	63,2a	(26-79a)	61,8a	(20-79a)	
Tamaño tumor					
<5 cm	4	(22,2)	26	(42,6)	
>5 cm	14	(77,8)	35	(57,4)	0,12
Pared gástrica					
T0-T1	3	(15,8)	9	(12,9)	
T2-T4	16	(84,2)	61	(87,1)	0,71
Bormann I	0	-	4	(6,6)	
Bormann II	2	(12,5)	19	(31,1)	
Bormann III	11	(68,8)	26	(42,6)	0,06[a]
Bormann IV	2	(12,5)	9	(14,8)	
Bormann V	1	(6,2)	3	(4,9)	
Linfonodos					
negativo	8	(42,1)	19	(37,3)	
positivo	11	(57,9)	32	(62,7)	0,71
Histología					
intestinal	13	(59,1)	51	(71,8)	
difuso	9	(40,9)	20	(28,2)	0,26

[a]Bormann III vs Bormann I, II, IV y V.

de células tumorales positivas a nivel nuclear (Figura 2) y los resultados se muestran en la Tabla 2. Se observa que sólo 3 (14,2%) de 21 tumores VEB+ presentaron acumulación de p53. Por el contrario, entre los tumores VEB-, 21 (36,8%) de 57 casos VEB- acumularon p53 (p=0,06). La determinación de Her2/neu se realizó en 20 casos VEB+ y 61 casos VEB-. Se consideró tinción positiva la tinción de membrana citoplasmática exclusivamente en las células tumorales (Figura 3). Los resultados se muestran en la Tabla 2. No observamos expresión de Her2/neu en ningún caso de CG cardial VEB+, y sólo en 3 (4,9%) casos VEB-.

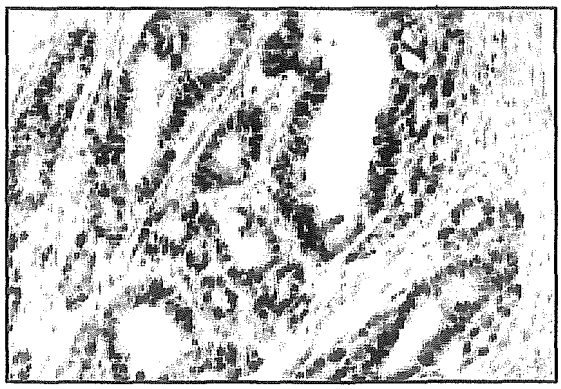

FIGURA 2. Inmunohistoquímica para proteína tumoral p53. Se observa tinción de p53 en el núcleo de células tumorales (40X).

Análisis de sobrevida. De 93 pacientes portadores de CG cardial, los datos de sobrevida fueron

Tabla 2. Correlaciones moleculares del cáncer gástrico cardial asociado al virus de Epstein-Barr

	VEB positivo n (%)	VEB negativo n (%)	p
p53			
negativo	18 (85,7)	36 (63,2)	
positivo	3 (14,3)	21 (36,8)	0,06
Her2/neu			
negativo	20 (100)	58 (95,1)	
positivo	0 0	3 (4,9)	0,17

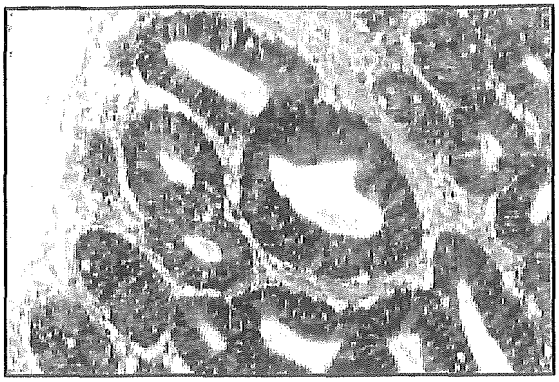

FIGURA 3. Inmunohistoquímica para oncogen c-erbB-2. Se observa tinción en la membrana citoplasmática de células tumorales (40X).

obtenidos en 77 casos, 18 VEB+ y 59 VEB-. Observamos una sobrevida global de 67% y 42% para los tumores VEB+ y VEB-, respectivamente. La tendencia en el riesgo relativo de morir fue 0,57 (95% IC=0,23 a 1,41; p=0,23) para los tumores VEB- con respecto a los tumores VEB+. Para comparar la probabilidad de sobrevida en tiempos específicos entre tumores EBV+ y EBV-, realizamos un análisis de función de sobrevida (Tabla 3), que nos muestra que los tumores EBV+ tienen una mejor sobrevida inicial, pero que estas diferencias tienden a desaparecer después de los 48 meses de seguimiento.

Tabla 3. Análisis de la función de sobrevida para el virus Epstein-Barr en cáncer gástrico cardial

Tiempo	VEB-	VEB+
1 mes	100%	100%
12 meses	78,1%	94,4%
24 meses	63,7%	88,8%
36 meses	57,1%	76,1%
48 meses	52,4%	69,8%
60 meses	52,4%	62,8%
72 meses	52,4%	62,8%

DISCUSIÓN

Varias líneas de evidencias apoyan la asociación entre VEB y CG. La demostración de monoclonalidad del genoma de VEB en casos de CG indica que la infección viral precede a la expansión neoplásica[30]. Por otra parte, la presencia de anticuerpos anti-VEB elevados en pacientes con CG asociado a VEB respecto a pacientes con CG-VEB negativo o sujetos controles[30-32], y la presencia del patrón histológico denominado *lace pattern*[33], característico de esta forma tumoral, son otras evidencias que apoyan esta asociación.

Previamente hemos identificado una fuerte relación entre VEB y CG de ubicación cardial[19]. Para caracterizar esta asociación, analizamos características clínico-patológicas, moleculares y de sobrevida en una serie de 93 casos de CG de ubicación cardial de los cuales 22 (22,6%) correspondieron al tipo asociado a VEB. Al comparar las características anátomo-patológicas, llama la atención la mayor frecuencia de presentación Bormann III en los tumores VEB+. Esta observación no ha sido descrita previamente, sólo Yanai et al[34] han señalado que la presentación endoscópica más frecuente del CG asociado a VEB sería la incipiente IIc. Por otra parte, ambos tipos histológicos de Lauren estuvieron representados en proporciones similares. Esta observación es contraria a la asociación descrita con el tipo "difuso"[19] y probablemente indica que la localización y el tipo histológico serían variables independientes en el CG asociado a VEB.

La proteína p53 es una de las más importantes en carcinogénesis gástrica[35]. Esta proteína es de ubicación nuclear, es activada en respuesta a daño celular y su inactivación se asocia al

desarrollo de neoplasias[36]. La inactivación, por mutaciones puntuales o complejos con proteínas virales estabiliza a p53, con aumento de su vida media, característica que permite el uso de la inmunohistoquímica para su detección[37]. Utilizando esta metodología, la frecuencia de acumulación de p53 varía entre 23% y 61%[37]. Nuestros resultados muestran una tendencia a una baja frecuencia de acumulación de p53 en tumores asociados a VEB, los cuales son concordantes con la literatura[38,39] aunque en estos trabajos no se hace referencia a la ubicación cardial. C-erbB2 es un oncogen que codifica para receptores de factores de crecimiento y está activado por amplificación génica[29]. La amplificación de c-erbB2 es considerado el factor pronóstico molecular más importante en CG, ya que se correlaciona con invasión serosa y linfática, metástasis hepática y peritoneal y menor sobrevida a 5 años[40]. Sin embargo, no hay estudios que analicen el rol de c-erbB2 en CG asociado a VEB y nuestros resultados no demuestran una relación entre ambos, al menos en ubicación cardial.

Con relación a sobrevida, aunque no observamos diferencias estadísticamente significativas, en la sobrevida entre los tumores VEB+ y VEB-, sí observamos una tendencia a mejor pronóstico en los tumores EBV+. Esta información es concordante con la descrita por van Beek et al[41], en población caucásica y por nuestro propio grupo en una serie de 192 CG japoneses[42]. Dado que la ubicación cardial se considera de peor pronóstico[5], la observación de una tendencia a mejor pronóstico en los tumores EBV+, sería sugerente de un potencial rol específico de EBV+ en la historia natural del cáncer gástrico cardial. El análisis de la función de sobrevida, indica que la presencia de EBV sería particularmente relevante en los primeros 48 meses de seguimiento de estos pacientes.

En resumen, nuestros resultados, aunque no significativos, muestran tendencias de asociaciones clínico-moleculares y de sobrevida del CG cardial asociado a VEB. Estos resultados aportan información adicional a la caracterización del rol de VEB en CG cardial, un forma emergente de CG.

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Environmental Factors Related to Epstein-Barr Virus-Associated Gastric Cancer in Japan

C. Koriyama[1], S. Akiba[1], Y. Minakami[2], Y. Eizuru[2]

Dept. of Epidemiology and Preventive Medicine[1], Div. of Oncogenic and Persistent Viruses[2], Center for Chronic Viral Diseases, Kagoshima University Graduate School of Medical and Dental Sciences; Kagoshima, Japan

Epstein-Barr virus (EBV)-encoded small RNA can be detected in about 1-17 % of gastric carcinomas. To elucidate the lifestyles and other factors related to the EBV-associated gastric carcinoma (EBV-GC), we interviewed 43 EBV-GC cases and 162 non EBV-GC cases in Kagoshima Prefecture, Japan from 1996-2001. We mainly focused on lifestyles predominant among men because of its male predominance. Although the prevalence of smokers in EBV-GC cases was higher than among non EBV-GC cases, the difference was not significant (P=0.131). Frequent drinking of coffee and high-temperature drinks, as well as frequent intake of salty and spicy foods, were more prevalent among EBV-GC cases, but only frequent intake of salty food showed a significant difference between EBV-GC and non EBV-GC cases (P=0.026). In addition, EBV-GC cases tended to be exposed to wood dust and/or iron filings (P=0.068) and tar (P=0.097). These findings, together with a high frequency of EBV-GC among remnant cancers after partial gastrectomy, suggest an association between mechanical injuries to the stomach membrane and the high frequency of EBV-GC. The present study also showed that EBV-GC cases tended to be elder brothers/sisters (P for trend =0.029) suggesting that age at primary infection with EBV may be older in EBV-GC cases than non EBV-GC cases.

Key Words: Epstein-Barr virus, Gastric carcinoma, Lifestyles, Wood dust, Birth order

An *in situ* hybridization (ISH) of Epstein-Barr virus-encoded small RNA (EBER), which became available in the early 1990's, elucidated that EBER can be detected in about 1-17 % of gastric carcinomas (1-5). Such an Epstein-Barr virus-associated gastric carcinoma (EBV-GC) has the uniform expression of EBNA-1, BARTs, and EBERs in all carcinoma cells in addition to the episomal monoclonality of the EBV genome (6), elevated serum antibodies against the EBV-related antigens (7,8), and the unique 'lace pattern' morphology in some early-stage-EBER-positive gastric adenocarcinomas (9). These features strongly suggest an important etiological role of EBV in the development of EBV-GC.

Another epithelial malignancy, that is well known for the EBV involvement, is the undifferentiated nasopharyngeal carcinoma (NPC). The association of EBV with a high incidence of NPC in southern China is strongly suspected. In most of the countries around the world, including southern China and Japan, EBV infection takes place in the early childhood (10). Since EBV infection can be seen worldwide, and most of the people in China become infected with EBV during adolescence, involvement of other factors are suspected in NPC development. Many studies indicated that high consumption of salted fish in early childhood is involved. The prevalence of EBV infection among adults is also nearly 100% in Japan, suggesting that other factors are also involved in the development of EBV-GC.

EBV-GC is known for its male predominance (11), predisposition to gastric fundic-gland region, and relatively high frequencies in the moderately- differentiated and the poorly-differentiated solid types than in other histological types (1-5). It is also known that the proportion of EBV-GC in remnant gastric cancers, occurring in the remaining part of the stomach after partial gastrectomy, is quite high (as high as 25%) (12,13). The high frequency of EBV-GC in remnant stomach cancer suggests the possibility that mechanical injuries of the stomach membrane are involved in the development of EBV-GC. In this context, the male

predominance of EBV-GC may also suggest the association of EBV-GC with mechanical injuries of the stomach membrane related to life-styles common among men.

In the present study, we interviewed EBV-GC and non EBV-GC cases to make comparisons between them with respect to environmental factors, including life-styles and other factors.

Materials and Methods

Subjects and Interview. We tried to identify EBV-GCs in 25 hospitals in Kagoshima city and its neighboring towns concurrently during the period between 1996 and 2001. Biopsy specimens were used for the screening of EBER status, and 43 EBV-GC cases were identified. Once an EBV-GC case was identified, we sought interview from the case during his/her hospital stay. In general, we tried to interview 2-4 non EBV-GC cases per week at two major hospitals, where the majority of EBV-GC cases were identified. In small hospitals, we sought interview from a patient with gastric carcinoma in the same hospital as the corresponding EBV-GC case. Although we did not have information on the EBER expression on those gastric carcinoma cases at the interview, none of those cases turned out to be EBER positive since the frequency of EBV-GC was low. In most of the cases, interview was not refused. Interview was successful in 162 non EBV-GC cases. From the present study, we excluded remnant cancer cases and multiple cancer cases. A structured questionnaire was used at the interview, and information obtained was on number of siblings, birth order, smoking history, drinking and dietary habits, and history of occupational exposure to tar and wood dust and/or iron filings.

Histology. Gastric carcinomas were classified according to the classification scheme of the Japanese Research Society for Gastric Cancer (14). Briefly, the histological patterns were considered as follows: well-differentiated tubular adenocarcinoma (tub1), moderately differentiated tubular adenocarcinoma (tub2), solid poorly differentiated adenocarcinoma (por1), non-solid poorly differentiated adenocarcinoma (por2), signet ring cell carcinoma (sig), and mucinous carcinoma (muc). According to Lauren classification (15), intestinal-type tumors include carcinomas with types tub1, tub2, and muc; diffuse-type tumors include carcinomas with types por1, por2, and sig. The location of a tumor, defined as the predominant location of the tumor, was divided into the following three categories: cardia or upper third part, middle part and antrum or lower third part according to the guidelines of the Japanese Research Society for Gastric Cancer (16).

ISH assay to detect EBER. The ISH assay of paraffin-embedded tissue samples obtained from the biopsy specimens was conducted using a digoxigenin-labeled EBER-1 oligonucleotide probe as described before (17). A case was considered to be EBER positive based on an intensive nuclear dark purple signal under microscopy. In every ISH assay, lymph node section from a patient with infectious mononucleosis and a sense probe for EBER-1 were used as positive and negative controls, respectively. In the present study, the case with EBER-1-positive tumor cells but not in the surrounding normal epithelial cells was determined to be an EBV-GC, and we defined the case with EBER-1-negative tumor cells as a non EBV-GC.

Statistical analysis. We examined the proportion of EBV-GCs using logistic regression analysis. Gender, age, and tumor location were included in logistic models as covariates. Maximum likelihood estimates of odds ratios (OR) and corresponding 95% confidence intervals (CIs) were calculated. The P value for trend of age was calculated using age as a continuous variable in a logistic model. All the P values presented were two-sided.

Results

Clinico-pathological characteristics of the study subjects are summarized in Table I. The EBV-GC showed a male predominance (P=0.008), predisposition to middle or upper part of the stomach (P=0.002), and high frequencies in tumors of diffuse type (P=0.022). There was no difference in the age distribution between EBV-GC and non EBV-GC cases.

The results of logistic analysis adjusting for the effects of gender, age, and tumor location are summarized in Tables II-IV. Although the ever-smokers (ex-smokers and current smokers) and frequent drinking of coffee was more prevalent among EBV-GC cases, the differences were not significant (P=0.131 and 0.344, respectively). Drinking of alcohol and 10 or more cups of Japanese green tea was not related to the frequency of EBV-GCs (Table II).

Table III summarizes the results of analysis on factors possibly related to stomach membrane injury. Fre-