

## Epstein-Barr Virus-associated Gastric Carcinoma in Japanese Brazilians and Non-Japanese Brazilians in São Paulo

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**The proportion of Epstein-Barr virus-associated gastric carcinoma (EBV-GC) was examined in 149 Japanese-Brazilian and 151 non-Japanese-Brazilian gastric-carcinoma cases using *in situ* hybridization (ISH) assay to detect EBV-encoded small RNA (EBER), and the results were compared with our referent Japanese data. We found that 4.7% of cases in Japanese Brazilians were EBER-positive. This frequency was slightly lower than that of the referent Japanese, among whom 6.2% of 2038 gastric-carcinoma cases were EBER-positive. On the other hand, the non-Japanese-Brazilian series showed a significantly higher proportion of EBV-GC (11.2%) than the referent group did ( $P=0.01$ ). Although EBV-GC was predominant in males among non-Japanese Brazilians (M/F=3.6,  $P=0.047$ ), as was the case in Japanese (M/F=2.7), Japanese Brazilians did not show such a male predominance. The sex-ratio difference between the Japanese Brazilians and Japanese was statistically significant ( $P=0.005$ ). In conclusion, the present study in Japanese Brazilians and Japanese yielded no evidence suggesting any change in the frequency of EBV-GC caused by migration, except the absence of male predominance, which was observed both in Japanese and non-Japanese Brazilians.**

Key words: Epstein-Barr virus — Gastric carcinoma — Japanese immigrants — Brazil

Epstein-Barr virus (EBV) is a human carcinogenic virus strongly suspected to cause Burkitt's lymphoma in Africa, nasopharyngeal carcinoma in southern China, and B-cell lymphoma in immuno-compromised hosts.<sup>1)</sup> In 1992, Shibata and Weiss reported that the EBV involvement could also be found in a small fraction of gastric-adenocarcinoma cases.<sup>2)</sup> They observed uniform presence of EBV-encoded small RNA (EBER) in tumor cells but not in the surrounding normal cells. A large-scale Japanese study conducted by Tokunaga *et al.* reported that 7% of gastric carcinoma cases were EBER-positive.<sup>3)</sup> Similar proportions of those EBV-associated gastric carcinomas (EBV-GCs) were found in Russia (8.7%),<sup>4)</sup> and Mexico (8.2%),<sup>5)</sup> while EBV-GC was more common in the United States (16%),<sup>2)</sup> Germany (18%)<sup>6)</sup> and Colombia (11.1%).<sup>7)</sup>

When the frequency of a disease is different in two countries, a migrant study can give important clues regard-

ing the etiology of that disease. A study of Americans with Japanese ancestry living in Hawaii, conducted by Shibata *et al.*, reported 10.2% of 187 gastric cancer cases to be EBV-associated.<sup>8)</sup> The observed percentage of EBV-GC was intermediate between the values in Japanese (7%)<sup>3)</sup> and Americans in Los Angeles (16%),<sup>2)</sup> suggesting that the frequency of EBV-GC cases may be affected by environmental factors. Another piece of evidence suggesting the involvement of environmental factors in EBV-GC is its male predominance. All the studies reported so far have shown that the proportion of EBV-GC in men is higher than that in women. In the present study, we examined the proportions of EBV-associated gastric-carcinoma cases in Brazilians with Japanese ancestry and non-Japanese Brazilians, and compared the results with the data obtained from a large-scale Japanese study in order to investigate whether or not the EBV-GC prevalence and its sex ratio vary among populations with different lifestyles and similar genetic backgrounds.

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**MATERIALS AND METHODS**

**Subjects** Three hundred and nine gastric cancer cases diagnosed at Hospital das clinicas, São Paulo University School of Medicine in São Paulo, Brazil, during the period 1949–1988 were recruited, and their demographic information and clinico-pathological data were retrieved from medical records. There were 149 Japanese Brazilians and 151 non-Japanese Brazilians (126 Caucasians and 25 blacks). We could not obtain the data on the birthplace of the Japanese Brazilians, but most of them were considered to be first or second generation Japanese immigrants on the basis of their ages. We excluded 9 cases from the analysis since there was no information on ethnicity. Thus, a total of 300 gastric cancer cases in São Paulo were used in the present analysis. As a reference group for the Japanese population, we used 2133 gastric cancers taken from 2064 gastric cancer cases in the present investigation. Among them, 269 cases were newly-diagnosed Japanese cases in Kagoshima Prefecture during the period 1993–1995, and 1795 gastric carcinoma cases were those used in the previous study reported by Tokunaga *et al.*<sup>3)</sup>

**Histology** Histological typing of tumors was conducted using the guidelines of the Japanese Research Society for Gastric Cancer.<sup>9)</sup> Briefly, the predominant histological structure of a carcinoma was classified as follows: well and moderately differentiated tubular adenocarcinoma (tub1 and tub2, respectively), solid and non-solid poorly differentiated adenocarcinoma (por1 and por2), signet-ring-cell carcinoma (sig), mucinous carcinoma (muc), and lymphoepithelioma-like carcinoma (LE). Papillary adenocarcinoma was included in the group of tub1. In the present study, we also used Lauren’s classification, consisting of two histological groups, i.e., intestinal and diffuse types. The intestinal type includes tub1 and tub2, and the diffuse type consists of por1, por2, sig, muc and LE.

Tumor subsite, which was defined as the predominant location of a tumor in the present study, was divided into the following four categories: cardia or upper third part, middle part, antrum or lower third part, and remnant.

**In situ hybridization assay to detect EBER** *In situ* hybridization (ISH) assay of paraffin-embedded tissue samples obtained from the main tumor was conducted using a digoxigenin-labeled EBER-1 oligonucleotide probe as described before.<sup>10)</sup> A case was considered to be EBER-positive based on a positive signal under microscopy. Lymph node sections from a patient with infectious mononucleosis and a sense probe for EBER-1 were used as positive and negative controls, respectively, in every ISH assay.

**Statistical analysis** Point estimates for the proportion of EBER-positive cases in each category and their corresponding 95% confidence intervals (95% CIs) were calculated by the normal-theory method. The exact method was used for the estimation of 95% CI when  $np(1-p)$  was less than five, where  $n$  is the number of subjects and  $p$  is the proportion of EBER-positive cases. Logistic analysis was conducted to compare the proportion of EBV-GC among gastric cancers. Sex, age and tumor subsite were always included in logistic models as independent variables. The sex ratio of EBV-GC was calculated by dividing the proportion of EBV-GC in men by that in women. Logistic analysis was also conducted to compare sex ratios in different ethnic groups. In the analysis, age and tumor subsite were always included in logistic models. All of the  $P$  values presented in the present study were two-sided.

**RESULTS**

**Demographic and clinical characteristics of study subjects** The present study examined gastric cancer diagnosed in 99 male and 50 female Japanese Brazilians, as well as 102 male and 49 female non-Japanese Brazilians (Table I). In Japanese gastric-carcinoma cases, there were 26 remnant gastric carcinoma cases, but there was no case in Brazilians. Since the proportion of EBV-GC in remnant gastric cancers was high (23.1%), we excluded those remnant gastric carcinoma cases from our analysis. Thus, we used a Japanese population consisting of 1293 men and 745 women as the reference group. Age distributions of

Table I. Frequency of EBER-positive Gastric Carcinoma Cases by Sex

Country of residence Ethnic group	Brazil									Japan		
	Japanese			Caucasian			Black			Japanese		
	EBER+/n	%	(95%CI)	EBER+/n	%	(95%CI)	EBER+/n	%	(95%CI)	EBER+/n	%	(95%CI)
Total	7/149	4.7	(1.3–8.1)	13/126	10.3	(5.0–15.6)	4/25	16.0	(4.5–36.1)	136/2038 <sup>a)</sup>	6.7	(5.6–7.8)
Male	3/99	3.0	(0.6–8.6)	11/84	13.1	(5.9–20.3)	4/18	22.2	(6.4–47.6)	105/1293	8.1	(6.6–9.6)
Female	4/50	8.0	(2.2–19.2)	2/42	4.8	(0.6–16.2)	0/7	0.0	—	22/745	3.0	(1.7–4.2)

a) Twenty-six remnant gastric carcinoma cases were excluded from the present analysis.

Brazilian and Japanese cases are shown in Table II. The referent Japanese series was the oldest among the four ethnic groups and its mean age was 6–14 years older than those of the three Brazilian groups.

**Distribution of EBV-GC by ethnic group** There were 7 (4.7%) and 17 (11.2%) EBV-GC cases in Japanese Brazilians and non-Japanese Brazilians, respectively, as shown in Table I. Caucasians in non-Japanese Brazilians numbered 126 (83.4%), and the others were black people. EBV-GC in Caucasians and blacks made up 10.3 and 16.0% of gastric carcinomas, respectively. When the effects of sex, age and tumor subsite were taken into account, there was no significant difference in the proportion of EBV-GC between Japanese Brazilians and the Japanese series ( $P=0.58$ ). However, the non-Japanese-Brazilian series showed a significantly higher proportion of EBV-GC (11.2%) than the referent group did ( $P=0.01$ ). Most EBV-GC cases of non-Japanese Brazilian were Caucasians. There were 4 EBV-GC cases in black males and none in black females.

**Distribution of EBV-GC by sex** The proportion of EBV-GC in men was higher than that in women among non-Japanese Brazilians (M/F=3.6,  $P=0.047$ ), as was the case in the referent Japanese series (M/F=2.7). On the other hand, Japanese Brazilians did not show such a male predominance. Using logistic models, we examined the difference of sex ratios in the Japanese Brazilians, non-Japanese Brazilians, and Japanese. The sex-ratio difference between the Japanese Brazilians and Japanese was statistically significant ( $P=0.005$ ), whereas the slight difference between non-Japanese Brazilians and Japanese was not ( $P=0.559$ ). Male predominance was observed in both Caucasian and black gastric-carcinoma cases.

**Distribution of EBV-GC by age and year of diagnosis** Table II shows the proportion of EBV-GC in three age groups. A weak age-dependent decrease of EBV-GC proportion was observed in our referent Japanese data ( $P=0.054$ ). Age dependence in the same direction as the referent was observed in Japanese Brazilians ( $P=0.190$ ) and non-Japanese Brazilians ( $P=0.870$ ). However, the age

Table II. Frequency of EBER-positive Gastric Carcinoma Cases by Age

Country of residence	Brazil									Japan		
	Japanese			Caucasian			Black			Japanese <sup>a)</sup>		
	EBER+/n	%	(95%CI)	EBER+/n	%	(95%CI)	EBER+/n	%	(95%CI)	EBER+/n	%	(95%CI)
Age group (years)												
-40	2/18	11.1	(1.4–34.7)	2/22	9.1	(1.1–29.2)	1/9	11.1	(0.3–48.2)	11/112	9.8	(4.3–15.3)
41–60	4/77	5.2	(1.4–12.8)	8/64	12.5	(4.4–20.6)	2/10	20.0	(2.5–55.6)	43/676	6.4	(4.5– 8.2)
61–	1/54	1.9	(0.05–9.9)	3/40	7.5	(1.6–20.4)	1/6	16.7	(0.4–64.1)	73/1237	5.9	(4.6– 7.2)
Mean age (SD)	55.9 (12.1)			53.8 (12.0)			48.5 (13.4)			62.1 (11.8)		

a) Thirteen cases had no data on age.

Table III. Frequency of EBER-positive Gastric Carcinoma by Tumor Site

		Japanese Brazilian			Non-Japanese Brazilian			Japanese		
		EBER+/n <sup>a)</sup>	%	(95%CI)	EBER+/n <sup>a)</sup>	%	(95%CI)	EBER+/n	%	(95%CI)
Male	Antrum	0/50	0.0	—	8/66	12.1	(4.2–20.0)	22/640	3.4	(2.0–4.8)
	Middle	1/14	7.1	(0.2–33.9)	1/3	33.3	(0.8–90.6)	66/513	12.9	(10.0–15.8)
	Cardia	0/4	0.0	—	1/4	25.0	(0.6–80.6)	19/196	9.7	(5.6–13.8)
Female	Antrum	3/26	11.5	(2.4–30.2)	0/29	0.0	—	8/370	2.2	(0.7–3.6)
	Middle	0/5	0.0	—	1/2	50.0	(1.3–98.7)	6/318	1.9	(0.4–3.4)
	Cardia	0/2	0.0	—	0/2	0.0	—	8/70	11.4	(4.0–18.9)
Total	Antrum	3/76	3.9	(0.8–11.1)	8/95	8.4	(2.8–14.0)	30/1010	3.0	(1.9–4.0)
	Middle	1/19	5.3	(0.1–26.0)	2/5	40.0	(5.3–85.3)	72/831	8.7	(6.8–10.6)
	Cardia	0/6	0.0	—	1/6	16.7	(0.4–64.1)	27/266	10.2	(6.5–13.8)

a) There was no data on site of tumor for 48 Japanese Brazilian and 48 non-Japanese Brazilian.

dependence was not statistically significant in any of the three ethnic groups. The year of diagnosis in the two Brazilian populations was about 20 years earlier than that in the Japanese series. The proportion of EBV-GC showed no evident trend in year of diagnosis (data not shown).

**Distribution of EBV-GC by subsite of tumor** When EBER-negative tumors were examined, tumor-subsite distribution was significantly different among the three ethnic groups ( $P < 0.001$ ) (Table III). The antrum was by far the most prominent tumor subsite in Brazilians with Japanese ancestry and non-Japanese Brazilians. In the referent Japanese series, the antrum was also the most predominant tumor subsite. However, what made the Japanese series different from Brazilians was the fact that middle-part tumors numbered only slightly fewer than antrum tumors.

Table III also shows that non-Japanese-Brazilian males had EBV-GC proportions higher than Japanese males did in every tumor subsite. The differences were not statistically significant, though ( $P$  values for antrum, middle, and cardia were 0.060, 0.355, and 0.539, respectively). All EBV-GC cases in non-Japanese-Brazilian males were Caucasians except 2 black males with a tumor in the antrum.

**Distribution of EBV-GC by histological type** When EBER-negative tumors were examined, Lauren's diffuse type was more common than Lauren's intestinal type in Japanese Brazilians and non-Japanese Brazilians, whereas the opposite was true in the referent Japanese data (Table IV). On the other hand, EBV-GC showed a predominance of the diffuse type in all three ethnic groups.

The proportion of EBV-GC in the diffuse-type tumors was slightly larger than that in the intestinal-type tumors in Japanese and non-Japanese Brazilians although the differences were not statistically significant. In our referent Japanese data, EBV-GC made up 7.5% and 4.8% of

diffuse- and intestinal-type tumors, respectively. The observed difference was mainly due to the high EBV-GC proportion in LE type, where 17 cases or 89.5% were EBER-positive. When LE carcinomas were excluded from diffuse-type tumors, the proportion of EBV-GC became 5.9%, which was not significantly different from that observed in intestinal-type tumors ( $P = 0.232$ , logistic analysis).

## DISCUSSION

In the present study, we obtained no evidence suggesting that the frequency of EBV-GC in Japanese Brazilians was different from that in our referent Japanese group. On the other hand, non-Japanese Brazilians had a proportion of EBV-GC as high as that reported in a US study.<sup>2)</sup> A high proportion was observed in all of the three tumor subsites, in both of the two Lauren's classification categories, i.e., diffuse and intestinal types, and in both Caucasians and blacks. Although the high proportion was limited to males, it was difficult to draw conclusions on female EBV-GC because of the paucity of female subjects in non-Japanese Brazilians.

Although the year of diagnosis in the two Brazilian populations was about 20 years earlier than that in the Japanese series, there was no evident relationship between the proportion of EBV-GC and year of diagnosis in either series ( $P = 0.32$  and  $0.21$  for Brazilian and Japanese series, respectively). Even when we limited the cases to those diagnosed in the same period from 1971 to 1990, we observed a significantly higher proportion of EBV-GC in non-Japanese Brazilians than that in the Japanese series ( $P = 0.02$ ). Furthermore, there was no significant difference in the frequency of EBV-GC between Japanese-Brazilians and the Japanese series ( $P = 0.52$ ).

Table IV. Frequency of EBER-positive Gastric Carcinoma by Histological Diagnosis

	Japanese Brazilian			Non-Japanese Brazilian			Japanese		
	EBER+/n	%	(95%CI)	EBER+/n	%	(95%CI)	EBER+/n <sup>a)</sup>	%	(95%CI)
Intestinal type	2/61	3.3	(0.4–11.3)	6/66	9.1	(2.2–16.0)	54/1132	4.8	(3.5–6.0)
tub1	0/19	0.0	—	1/25	4.0	(0.1–20.4)	8/534	1.5	(0.5–2.5)
tub2	2/42	4.8	(0.06–16.2)	5/41	12.2	(4.1–26.2)	46/598	7.7	(5.6–9.8)
Diffuse type excluding LE	5/87	5.7	(1.9–12.9)	11/84	13.1	(5.9–20.3)	55/938	5.9	(4.4–7.4)
por1	4/33	12.0	(3.4–28.2)	7/35	20.0	(6.7–33.3)	42/420	10.0	(7.1–12.9)
por2	1/42	2.4	(0.06–12.6)	4/32	12.5	(3.5–29.0)	9/332	2.7	(1.0–4.5)
sig	0/4	0.0	—	0/3	0.0	—	2/121	1.7	(0.2–5.8)
muc	0/8	0.0	—	0/14	0.0	—	2/65	3.1	(0.4–10.7)
LE	—/0	—	—	—/0	—	—	17/19	89.5	(66.9–98.7)
Malignant lymphoma	0/1	0.0	—	0/1	0.0	—	—/0	—	—
Others	—/0	—	—	—/0	—	—	0/2	0.0	—

a) Seventeen cases had no data on histological diagnosis.

The proportion of EBV-GC in Japanese-American cases living in Hawaii (10.2%)<sup>8)</sup> was intermediate between Japanese (7%)<sup>3)</sup> and American cases in Los Angeles (16%).<sup>2)</sup> As opposed to the study of Japanese Hawaiians, the present study gave no evidence suggesting any change in the frequency of EBV-GC caused by migration. This discrepancy between EBV-GC in Japanese living in Hawaii and Brazil may be explained by the difference of tumor-subsite distribution in the two groups. Kamineni *et al.* investigated the incidence of gastric carcinoma by tumor subsite in Asian migrants to the US and their descendants, and Japanese Americans were revealed to have a high prevalence of tumors in gastric cardia or cardio-esophageal junction (6.4%).<sup>11)</sup> This result suggested that the high frequency of EBV-GC in Japanese Hawaiians was because of the high prevalence of tumors occurring in cardia. However, we could not examine this possibility since tumor-subsite specific proportions of EBV-GC were not reported in the US study.

Brazil has the largest population of Japanese immigrants in the world, and approximately 1 200 000 Japanese immigrants and their descendants are living in Brazil. In 1908, the first immigration from Japan to Brazil started, and about 250 000 Japanese had moved to Brazil by 1978. According to the recent report by Cardoso *et al.*, there has been an evident change in the lifestyle of Japanese Brazilians over the years, and current diets, especially among second-generation Japanese Brazilians, are similar to those of Europeans.<sup>12)</sup> On the basis of the reported changes of diet in Japanese Brazilians, cancer site distribution in Japanese Brazilians was expected to become similar to those in the US and west European countries. However, the magnitude of decrease in stomach cancer incidence among Japanese Brazilians was smaller than that of Japanese Americans in Hawaii; stomach cancer is still the predominant malignant disease among the first generation of Japanese Brazilians in São Paulo.<sup>13)</sup> The increases of cancers of the prostate and breast among Japanese Brazilians were also less marked than those of Japanese Americans in Hawaii. Those observations suggested that Japanese immigrants to São Paulo might have a tendency to retain their native dietary habits. Indeed, the proportion of first-generation immigrants in Japanese Brazilians was higher than that in Japanese Americans in Hawaii. We could not obtain information on birthplace of Japanese Brazilians in the present study. However, most cases were likely to be first- or second-generation Japanese Brazilians judging from their ages. Thus, the reason why the proportion of EBV-GC did not increase among Japanese immigrants in São Paulo may be that they have not changed their dietary habits so much as Japanese Hawaiians did.

The tumor-subsite distribution of gastric carcinoma was significantly different among the three ethnic groups. Tumors occurring in the antrum were dominant in all

groups (75.2%, 89.6%, and 47.9% in Japanese Brazilian, non-Japanese Brazilian, and Japanese, respectively), but tumors occurring in middle and cardia were more frequently observed in Japanese cases. The difference between the Japanese and Brazilian series could not be explained by difference in year of cancer diagnosis. Even when we limited the cases to those diagnosed from 1971 to 1990, the difference of tumor-subsite distribution in the two populations was still significant (data not shown). Of interest was the finding that Japanese Brazilians showed a higher proportion of gastric carcinoma in the antrum than Japanese did. Since Western countries showed predominance of gastric carcinoma in cardia (40–50%),<sup>14–16)</sup> and a Western-style diet decreased the risk of gastric cancer in antrum,<sup>17)</sup> our findings support the conclusion that Japanese Brazilians still retain Japanese traditions, to some degree, in their dietary habits. EBV-GC in Japanese and non-Japanese Brazilians was slightly more common in the diffuse type than in the intestinal type, as was the case in the referent Japanese data. However, the number of cases in each subgroup of the Brazilian series was too small to draw any definitive conclusion in this regard.

Male predominance of EBV-GC, which was evident in non-Japanese Brazilians, was not observed in the Japanese Brazilians in the present study. Male predominance has been consistently observed in the studies reported so far. The most marked ones were the sex ratio of 7.0 in Caucasians living in Los Angeles<sup>2)</sup> and 6.2 in Russians.<sup>4)</sup> To date, an exception is the Mexican study, reporting a sex ratio of a mere 1.1.<sup>5)</sup> At this moment, we have no good explanation for the absence of male predominance in Japanese Brazilians, and the wide range of the sex ratio reported in the literature. Some environmental factors to which males are predominantly exposed in most countries may be one of the possible explanations.

According to a study by Candeias and Pereira, more than 80% of Brazilians were infected with EBV by the time they became adults.<sup>18)</sup> This prevalence was lower than that observed in a Japanese normal population.<sup>19)</sup> Thus, the high frequency of EBV-GC in non-Japanese Brazilians could not be explained by the prevalence of EBV infection among the normal population.

Since LMPs, which are important oncoproteins of EBV, are not expressed on tumor cells of EBV-GC,<sup>20)</sup> it is still unclear whether EBV plays an important role in the etiology of EBV-GC. However, EBV-GC is known for the episomal monoclonality of the EBV genome and elevated antibodies against EBV-related antigens.<sup>21, 22)</sup> Levine *et al.* reported that EBV-GC patients have significantly high antibody titers against EBV capsid antigen more than 5 years preceding their diagnoses.<sup>23)</sup> In addition, unique morphologic features have been observed in some early-stage EBV-GCs, characterized by lace patterns of branching and lymphocytic infiltration in and around the carci-

noma nest in mucosa.<sup>24)</sup> These findings appear inconsistent with the hypothesis that the EBV persists as a “passenger” in gastric carcinoma cells.

In conclusion, EBV-GC in Japanese Brazilians did not show evident differences from that in our referent Japanese data except for the absence of male predominance of EBV-associated tumors, which has been observed in most of the previous studies as well as in the non-Japanese Brazilians of the present study. Further studies are necessary to draw any definitive conclusion because the results obtained by the present study were based on small numbers of cases in Brazilian populations.

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