Association of a distinctive strain of Epstein-Barr virus with gastric cancer

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Epstein-Barr virus (EBV) has been linked to gastric carcinoma (GC) with worldwide geographical variations attributable to types and variants of EBV. Here, we compare EBV strains between EBVaGC and healthy donors in Latin America, a high frequency area for EBVaGC. Tumor samples from 73 EBVaGC cases and throat washings from 329 healthy adults were examined for types 1 and 2 EBV and polymorphism at BamHI-F and BamHI-W1/I1 boundary regions and XhoI restriction site in LMP1 gene. Type 1 and prototype F of BamHI- F polymorphism accounted 59 (81%) and 69 (95%) of EBVaGC cases and 257 (78%) and 267 (81%) of healthy donors, respectively. Types I and "i" of BamHI W1/I1 polymorphism accounted 2 (3%) and 62 (85%) of EBVaGC and 85 (26%) and 170 (52%) of healthy donors, respectively ($p<0.001$). XhoI+ and − polymorphism accounted 60 (82%) and 4 (5%) of EBVaGC and 142 (43%) and 92 (28%) of healthy donors, respectively ($p<0.001$). Cosegregation analysis demonstrated that most of the 62 type "i" EBVaGC cases harbor XhoI+ strain (81%). However, among 143 type "i" healthy adults, both XhoI polymorphism were present in relatively similar frequencies (XhoI+ 58% and XhoI− 42%) (OR 9.0; 95% CI 1.2–69). Our findings are against to the proposed hypothesis that EBV strains are geographically but not disease-restricted. We conclude that most of the EBVaGC cases harbor a distinctive EBV strain (type "i"/XhoI +), but in healthy donors, this strain was as common as other strains. This finding is contrary to the proposed hypothesis that EBV strains are geographically but not disease-restricted and identified a healthy population group that share the same strain that predominate in EBVaGC cases.

Key words: gastric cancer; Epstein-Barr virus; genotypes

Epstein-Barr virus (EBV), an ubiquitous human herpesvirus, causes infectious mononucleosis after primary infection in young adults, and is closely associated with lymphoid neoplasms, such as B-cell lymphomas among immunosuppressed patients, Burkitt lymphoma, and Hodgkin lymphoma Hodgkin's disease.[1,2] EBV has also been associated with epithelial malignancies like nasopharyngeal carcinoma (NPC), lymphoepitheliomas of several organs,[3] and during the last decade, it has been linked to gastric carcinoma (GC).[4] This latter association has been evidenced by the presence of uniform expression of EBV-encoded small RNA type-1 (EBER-1) in all GC tumor cells,[5] the detection of monoclonal EBV episomes in GC cells[6] and the elevation of serum antibodies against viral capsid antigen in EBV-associated GC (EBVaGC) patients but not in EBV-negative GC patients or healthy controls.[6–8] Worldwide, EBVaGC represents about 10% of GC;[4] however, the frequency of this association varies from country to country and an inverse correlation between GC mortality and frequency of EBVaGC has been found;[9] *e.g.*, countries with a low GC mortality rate, such as the U.S.A. and Germany,[10] showed the highest frequencies of EBVaGC (16–18%)[11,12] and in countries with a high GC mortality rate, like Japan and China,[10] the proportion of EBVaGC accounts only for 6–7%.[13,14] In Latin America, we reported a frequency as high as 17% of EBVaGC, with a significant association to cardia location and diffuse histology.[15,16]

The 2 major types of EBV, type 1 and 2, differ in the sequence of EBNA-2, 3A, 3B, 3C and LP genes and in their capacity to transform B-lymphocytes into a state of continuous proliferation.[17] Type 1 EBV is the predominant strain in Western and Asian countries while type 2 EBV is frequently found in Africa.[18,19] In addition, 3 major variants of EBV have been identified based on restriction fragment length polymorphism (RFLP) of BamHI and XhoI restriction endonuclease map of the prototype B95.8 genome.[20–23] At BamHI-F region, the prototype F has a worldwide distribution but "f" variant, featured by the presence of an extra BamHI site, is found only in Southern China where it is associated with NPC.[24] The polymorphism at BamHI W1/I1 boundary region identifies 2 types, type I and "i". Type I lacks the BamHI site and predominates among healthy people and EBV-associated diseases in Japan and China.[20,25,26] Type "i", which keeps BamHI restriction site prevails in healthy donors and EBV-associated disease in Western countries.[21] Finally, the lack of XhoI restriction site at exon 1 of the LMP1 gene defines the XhoI − genotype, which is common in Asia[27] while the XhoI + variant is frequently observed in Western countries.[22]

Taken together, these observations suggest that geographical distribution of EBVaGC might be explained by different EBV genotypes or variants around the world. In this study, we compare types and variants of EBV among EBVaGC cases and healthy adults in Latin America.

Material and methods

Specimens

We examined formalin-fixed and paraffin-embedded tumor samples of 73 EBVaGC cases (44 from Chile and 29 from Colombia) and throat washing samples collected from 329 healthy adults (140 from Chile and 189 from Colombia) by gargling with 15 ml of phosphate-buffered saline. In addition, 5 EBVaGC cases in which both tumor samples and throat washing gargles were also available were examined. The EBV expression status of the EBVaGC cases was examined previously[15,16] using *in situ* hybridization with oligonucleotide probes specific for the EBER-1 gene as described in detail elsewhere.[28] The Institutional Review Board of the San Borja-Arriaran Hospital, Santiago, Chile and the Institutional Review Board of the Faculty of Health, Universidad del

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TABLE I – LIST OF PRIMERS AND PROBES USED IN THE PRESENT STUDY

	Sequence	Type by probe or size after RE[1] digestion	Reference
EBNA-3C primers			
Sense	5′-AGAAGGGGAGCGTGTGTTGT-3′		
Antisense	5′-GGCTCGTTTTTGACGTCGGC-3′		Sample *et al*. (1990)
Probes			
Type A	5′-GAAGATTCATCGTCAGTGTC-3′	153 bp[2]	
Type B	5′-CCGTGATTTCTACCGGGAGT-3′	246 bp	
Bam-HI-F primers			
Sense	5′-TCCCACCTGTTACCACATTC-3′	Prototype F = 198 bp	Lung *et al*. (1994)
Antisense	5′-GGCAATGGGACGTCTTGTAA-3′	Variant "f" = 127+71 bp	
BamHI-W1/I1 primers			
Sense	5′-ACCTGCTACTCTTCGGAAAC-3′	Type I = 205 bp	Lung *et al*. (1994)
Antisense	5′-TCTGTCACAACCTCACTGTC-3′	Type "i" = 130+75 bp	
XhoI site in LMP1 gene primers			
Sense	5′-AACAGTAGCGCCAAGAGGAG-3′	XhoI− = 113 bp	Sandvej *et al*. (1997)
Antisense	5′-ATGGAACACGACCTTGAGAGG-3′	XhoI+ = 67+46 bp	

[1]Restriction enzyme.–[2]Base pairs.

Valle, Cali, Colombia approved this study and all healthy individuals as well as EBVaGC gave informed consent.

Preparation of DNA

Cellular material from throat washing was collected by centrifugation at 22,000*g* for 40 min and the resulting pellet was resuspended in 100 μl of extraction buffer (1 M Tris, pH 8.0, 50 mM EDTA, 0.5% Tween 20) with 100 μg/ml Proteinase K. After overnight incubation at 37°C and boiling at 100°C for 10 min for Proteinase K inactivation, samples were subjected to phenol–chloroform extraction and ethanol precipitation. Finally, DNA was dissolved in 40 μl of 10 mM Tris-HCl, pH 8.0, 1 mM EDTA (TE) buffer and kept at −30°C until amplification. Formalin-fixed, paraffin-embedded archival material was cut in 5 μm slices, treated with xylene and ethanol and centrifuged at 22,000*g* for 20 min, and the resulting pellet was resuspended in 100 μl of extraction buffer as described earlier.

Primers and probes

Primers and probes used in this study are shown in Table I. For distinguishing type 1 and 2 EBV strains, we used primers and probes described by Sample et al.[29] These probes recognized divergent sequences in the U2 region encoding EBNA-3 gene[30] and produced 153-and 246-bp, respectively. The BamHI-F region was amplified with primers described by Lung et al[20,31] that yield a 198-bp fragment. To distinguish the prototype F from the "f" variant, the 198-bp fragment was digested by BamHI restriction enzyme to yield a 198-bp fragment in the case of the F prototype and 127-bp and 71-bp fragments in the case of "f" variant. A 205-bp fragment of the BamHI-W1/I1 boundary region was amplified using the primer pair described by Lung et al.[20,31] Type I, 205-bp fragment, and type "i", 130- and 75-bp fragments, were determined by BamHI restriction enzyme digestion. Analysis of XhoI restriction site polymorphism in exon 1 of LMP1 gene was performed with a set of primers to produce a 113-bp amplified fragment.[32] Digestion with XhoI restriction enzyme resulted in 67-and 46-bp fragments for the XhoI + type and the undigested 113-bp PCR product indicates the XhoI − type. The cell line B95-8 served as positive control for type 1, prototype F, type I and XhoI + virus. The cell lines AG786 and Akata served as positive controls for type 2 and XhoI − virus, respectively. The cloned BamHI-"f" and BamHI-"i" DNA fragments served as positive controls for the "f" variant and type "i", respectively. The MOLT-4 cell line infected with human herpesvirus 6 served as negative control.

Polymerase chain reaction

Polymerase chain reaction (PCR) was performed with 2 μl of DNA in a 25 μl reaction mixture containing 10 mM Tris-HCl, pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$, 200 uM dNTP, 0.5 μM of each primer and 1.25 U Taq Polymerase (Invitrogen Corp., CA). The amplification profile for EBNA-3 gene, the BamHI-F region and the BamHI-W1/I1 boundary region amplification were 1 cycle at 95°C for 5 min, followed by 40 cycles of 92°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec. The program ended with 5 min at 72°C. For XhoI restriction site amplification, the profile was 1 cycle at 95°C for 5 min, followed by touchdown PCR [6 touchdown cycles at 94°C for 30 sec, 66°C for 30 sec with a decrease of 1°C each cycle, and 72°C for 30 sec, followed by 39 cycles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec], with a final extension at 72°C for 5 min.

Southern blot analysis

Specificity of the PCR reactions for type 1 and 2 EBV strains was confirmed by southern blot hybridization with specific probes. After 2% agarose gel electrophoresis, the electrophoretic pattern was transferred to a Hybond N+ nylon membrane (Amershan, Aylesbury, UK) by capillary blotting in 0.4 N NaOH solutions. Membranes were prehybridized with hybridization buffer and each probe was labeled with peroxidase using ECL direct labeling kit (Amersham, Aylesbury, UK). After adding the probe, hybridization was carried out overnight at 42°C. The hybridization signal was detected using ECL detection kit (Amershan, Aylesbury, UK) according to manufacturer's instructions.

Restriction fragment length polymorphism analysis

To perform an analysis of RFLP at BamHI-F region, BamHI-W1/I1 boundary region and XhoI restriction site, amplified PCR products were purified by phenol/chloroform extraction followed by ethanol precipitation with glycogen carrier. DNA pellets were resuspended in 50 μl of distilled water and 5 μl aliquots were digested of either BamHI, for BamHI-F and BamHI-W1/I1 boundary region RFLP, or XhoI, for the XhoI restriction site polymorphism. Restriction enzyme digestion was performed in 20 μl volumes with 10 U. of restriction enzyme for overnight according to manufacturer's instructions (Invitrogen Corp., CA). Resultant products were electrophoresed through 8% polyacrylamide gel in TBE (45 mmol/l Tris-Borate) and visualized by silver-staining method.[33–35]

Cloning and sequencing DNA

Amplified XhoI restriction site products from 5 EBVaGC cases were purified from low melting point agarose gels and cloned into the pGMET vector, using the Wizard PCR prep kit and pGMET cloning methods (Promega Corp, WI). Before precipitation for sequencing, plasmids were checked for correct insert size by PCR.

TABLE II – EPSTEIN-BARR VIRUS GENOTYPING IN EPSTEIN-BARR-VIRUS-ASSOCIATED GASTRIC CARCINOMA AND THROAT WASHING OF HEALTHY ADULTS

	EBVaGC[1] (*N* = 73)		TW[2] (*N* = 329)		*p* value[3]	Age-adjusted *p* value[4]
	N	%	*N*	%		
Gender						
Female	14	19	173	53	<0.001	–
Male	55	75	156	47		
Unknown	4	5	0	0		
Mean age (SD)	58.1	(14)	29.5	(10)	<0.001	–
Range	19–83		18–66			
EBNA-3C						
Type 1	59	81	257	78	0.045	0.222
Type 2	11	15	22	7		
NA[5]	3	4	50	15		
BamHI-F						
Prototype F	69	95	267	81	0.037	0.053
"f" variant	0	0	17	5		
NA[5]	4	5	45	14		
BamHI-W1/I1						
Type I	2	3	85	26	<0.001	<0.001
Type "i"	62	85	170	52		
NA[5]	9	12	74	22		
XhoI site						
−	4	5	92	28	<0.001	<0.001
+	60	82	142	43		
NA[5]	9	12	95	29		

[1]Epstein-Barr-virus-associated gastric carcinoma.–[2]Throat washing of healthy adults.–[3]*p* values were obtained by χ^2 test.–[4]*p* values were obtained by exact method.–[5]No amplified fragment.

A small sample of each clone was boiled in 10 µl water and 1 µl aliquot was amplified using T3 and T7 primers (Promega Corp, WI) under standard conditions using 30 cycles of PCR with annealing temperature of 55°C. Of the products, 5 µl was checked on agarose gel, and 5 µl aliquots of the remainder were used for sequencing. Clones were bidirectionally sequenced through cycle sequencing, using the Big Dye Terminator kit (Perkin Elmer, CT) on the automated ABI Prism 310 sequencer (Applied Biosystems, CA). Clones were sequenced at least 2 times to ensure sequence fidelity.

Statistical analysis

We conducted a case-control comparison for EBV strains between EBVaGC cases and healthy donors by using χ^2 test or two-sided Fisher's exact test. The results were considered to be statistically significant at a P of less than 0.05. Logistic regression analysis was conducted to compare the association between BamHI W1/I1 boundary and XhoI restriction site polymorphism. Exact P values and estimation of common odds ratios were obtained using stratified contingency tables by age (<30, 30–49 and 50<=). Statistical analyses were conducted using the EPICURE package of statistical programs for analysis of epidemiological data (Hirosoft, WA) or StatXact 4 (CYTEL Software Corporation, MA).

Results

We examined 73 EBVaGC and 329 healthy adults. Their age and gender distributions were different from each other as shown in Table II, however, neither gender or age affected the distribution of EBV subtype. Therefore, it was concluded that the gender or age difference between EBVaGC cases and healthy donors should not affect the comparisons in terms of EBV subtype distributions.

EBV type (type 1 and 2)

The amplification of U2 region encoding EBNA-3 gene was successful in 70 out of 73 (96%) EBVaGC and 279 out of 329 (85%) healthy donors. The distribution of types 1 and 2 between case and healthy donors groups is summarized in Table II. Type 1 accounted 59 (81%) of 73 EBVaGC and 257 (78%) of 329 healthy donors ($p = 0.045$). This difference was not statistically significant when corrected for age distribution ($p = 0.22$). The distribution of type 1 and 2 in the 2 countries did not differ significantly.

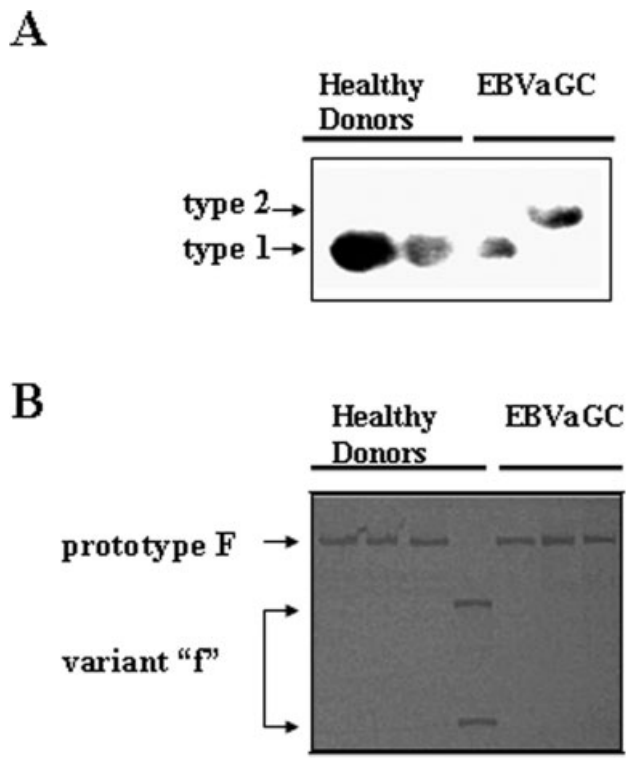

FIGURE 1 – Gentoypes of Epstein-Barr virus in Healthy donors and Epstein-Barr-associated gastric carcinomas (EBVaGC). (*a*) Southern blot analysis after PCR amplification and hybridization with specific probes for type 1 and 2 strains. (*b*) Polyacrylamide gel after PCR amplification and digestion with BamHI restriction enzyme for RFLP at BamHI-F region.

Representative examples of EBV types 1 and 2 in EBVaGC and healthy donors are shown in Figure 1*a*.

BamHI-F region (types F and "f")

The BamHI-F region could be amplified in 69 (95%) EBVaGC and in 284 (86%) healthy adults. The distribution of prototype F and "f" variant between EBVaGC and healthy adults is summarized in Table II. All of the 69 EBVaGC harbor the prototype F EBV. However, among healthy adults, prototype F and "f" variant were found in 267 (81%) and 17 (5%), respectively. This difference in cases and controls was statistically significant ($p = 0.037$), but not in age-corrected distribution ($p = 0.053$). Among 267 prototype F EBV, types 1 and 2 numbered 227 and 19, respectively. In the remaining 21 specimens, EBNA-3 region could not be amplified. Similarly, all but 1 of the 17 "f" variants obtained from healthy donors were identified as type 1 (data not shown). The distribution of prototype F and variant "f" in the 2 countries did not differ significantly. Representative examples of BamHI-F RFLP in healthy donors and EBVaGC are shown in Figure 1*b*.

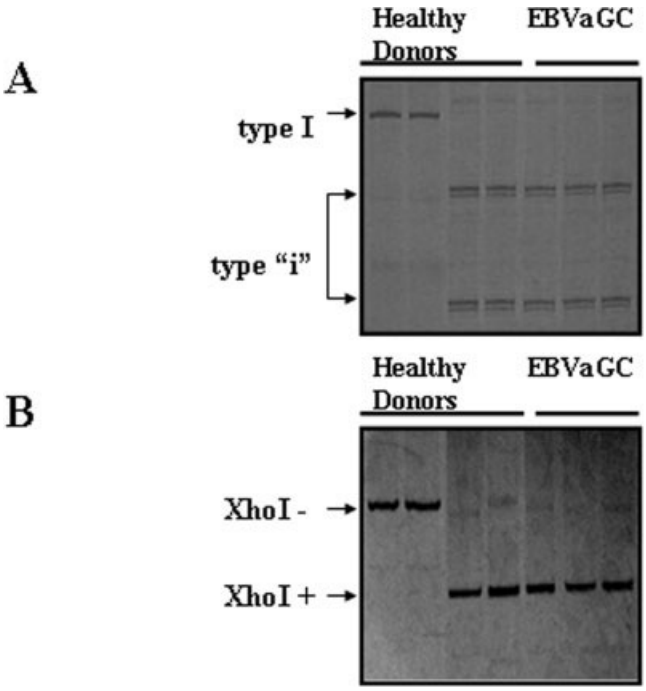

FIGURE 2 – Gentoypes of Epstein-Barr virus in Healthy donors and Epstein-Barr-associated gastric carcinomas (EBVaGC). (*a*) Polyacrylamide gel after PCR amplification and digestion with BamHI restriction enzyme at BamHI W1/I1boundary region. (*b*) Polyacrylamide gel after PCR amplification and digestion with XhoI restriction enzyme at XhoI site polymorphism. The second fragment after XhoI digestion (46-bp fragments) for the kept-type is not seen in the gel.

BamHI W1/I1 boundary (types I and "i")

The BamHI-W1/I1-boundary restriction site was successfully amplified in 64 (88%) of 73 EBVaGC and in 255 (78%) of 329 healthy adults. The distribution of types I and "i" between EBVaGC and healthy adults is summarized in Table II. Only 2 (3%) EBVaGC harbor type I EBV while 62 (85%) out of 73 EBVaGC harbor type "i" EBV. Among healthy adults, type I and "i" were found in 85 (26%) and 170 (52%), respectively. This difference was highly significant ($p < 0.001$). Among 170 type "i" strain, types 1 and 2 numbered 149 and 11, respectively. In the remaining 10, EBNA-3 region could not be amplified. Among 85 type I healthy donors, 73 were type 1, 5 were type 2 and EBNA3A could not be amplified in the remaining 7 subjects. The distribution of type I and "i" in both countries did not differ significantly. Representative examples of type I and "i" in EBVaGC and healthy donors are shown in Figure 2*a*.

XhoI restriction site polymorphism at exon 1 of the LMP1 gene

A successful analysis of XhoI restriction site polymorphism was performed in 64 (88%) of 73 EBVaGC and in 234 (71%) of 329 healthy controls. The distribution of XhoI + and − between EBVaGC and healthy adults is summarized in Table II. Sixty (82%) out of 73 EBVaGC harbor XhoI + EBV and only 4 (5%) individuals harbor type XhoI −. However, among healthy adults, XhoI + and − were found in 142 (43%) and 92 (28%), respectively. This difference was highly significant ($p < 0.001$). The distribution of XhoI + and − in both countries did not differ significantly. Representative examples of XhoI + and − in EBVaGC and healthy donors are shown in Figure 2*b*.

Cosegregation of XhoI restriction site polymorphism with BamHI *W1/I1 boundary RFLP*

Since it is known that XhoI restriction site polymorphism co-segregate with the BamHI W1/I1 boundary RFLP,[21] we next analyzed the frequency of co-segregation between these 2 polymorphism in EBVaGC and healthy donors (Table III). Among 62 type "i" EBVaGC, 59 (95%) harbor XhoI + strain and a recombinant type "i"/XhoI − strain was found in 3 (5%) individuals. Among 143 type "i" healthy adults, XhoI + type was found in 83 (58%) cases and the recombinant strain type "i"/XhoI − was present in 60 (42%) of subjects. Among type I EBVaGC, XhoI + and − was present in 1 patient respectively. In 69 type I healthy donors, XhoI + and − was found in 46 (67%) and 23 (33%) of individuals, respectively. The proportion of type "i"/XhoI + in EBVaGC was significantly higher than the proportion of 58% observed among healthy donors (Odds ratio 9.0; 95% Confidence interval 1.2–69, Table III)

Correlations of EBV genotypes in both tumor samples and throat washing from EBVaGC cases

In 5 additional EBVaGC cases, both tumor samples and throat washing were available for genotyping analysis. Genotyping analysis of these cases reveals the presence of unique strain type 1, type F, type "i" and XhoI + in tumor sample. Corresponding throat washing of these cases reveals same genotype in 4 out of 5 cases. The remaining case was type 1, type F, type "i" but XhoI − in gargles. Representative examples are shown in Figure 3.

TABLE III – COSEGREGATION ANALYSIS OF BamHI-W1/I1 BOUNDARY REGION AND XhoI RESTRICTION SITE IN LMP1 GENE IN EPSTEIN-BARR-VIRUS-ASSOCIATED GASTRIC CARCINOMA AND THROAT WASHING OF HEALTHY ADULTS

BamHI-W1/I1	XhoI site	EBVaGC[1]	TW[2]	OR (95% CI)[3]	Age-adjusted OR (95% CI)[4]
I	−	1	23	1.0 (reference)	1.0 (reference)
	+	1	46	0.5 (0.01–40.9)	0.58 (0.004–78.1)
i	−	3	60	1.2 (0.09–63.0)	0.85 (0.04–55.5)
	+	59	83	16.3 (2.5–685)	Inf (5.9 to +inf)

[1]Epstein-Barr-virus-associated gastric carcinoma.–[2]Throat washing of healthy adults.–[3]OR and 95% CI were calculated by logistic regression model.–[4]OR and 95%CI were obtained by exact method.

Sequencing of XhoI restriction site polymorphism in cases of EBVaGC

Finally, the presence of the distinctive XhoI + strain in EBVaGC cases was confirmed by sequence of XhoI region in 5 cases of EBVaGC. All 5 cases show the presence of XhoI restriction site. Representative sequence data is shown in Figure 4.

Discussion

In the present study, we conducted an EBV genotyping analysis in EBVaGC and healthy adults in 2 Latin American countries with high incidence and mortality rate for gastric cancer. Our results show that a distinctive viral genotype is present in EBVaGC, characterized as type "i" at the BamHI W1/I1 boundary region and XhoI + at XhoI restriction site polymorphism in exon 1 of the LMP1 gene. However, among healthy adults, these polymorphism were as common as other 3 genotypes (type I/XhoI − and the recombinants type "i"/XhoI − and type I/XhoI +). These observed differences cannot be attributed to detection rates of EBV, since our detection rates were comparable or even better than that reported by other researchers.[24,36–38] Thus, these differences suggest that a particular polymorphism (type "i"/XhoI +) is predominant in EBVaGC eventhough several strains are found in healthy donors. In addition, although in a small subset of cases, most of EBVaGV harbour the same strain in both stomach and throat washings. Taken together, our findings are similar to that of NPC in Southern China, where the presence of the "f" variant was found in most NPC cases and in a small proportion of healthy Chinese.[24] Additionally, our findings suggest that the proposed hypothesis that EBV strains are geographically but not disease-restricted[22] might not be true for EBVaGC in Latin America.

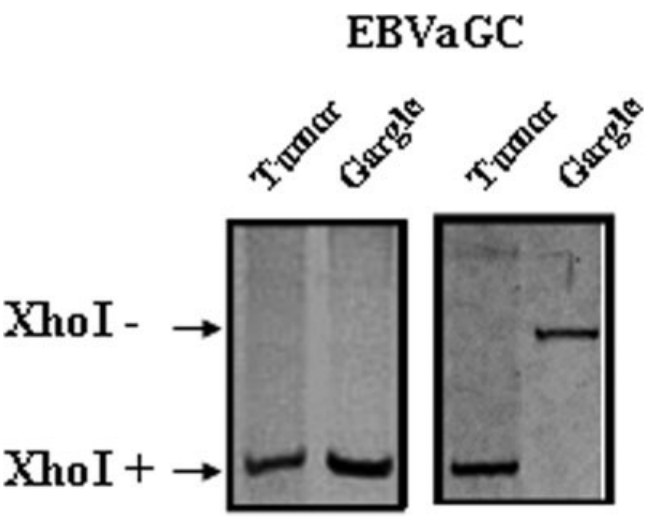

FIGURE 3 – Polyacrylamide gel after PCR amplification and digestion with XhoI restriction enzyme at XhoI site polymorphism in tumor and throat washing of Epstein-Barr-associated gastric carcinomas (EBVaGC).

The finding of a distinctive genotype in EBVaGC cases in comparison with healthy donors suggest that the expression of particular EBV gene(s) with transforming capacity might be encoded in the vicinity of BamHI W1/I1 boundary and XhoI restriction site polymorphism. There are at least 3 candidate genes involved in transformation and immortalization located in this region. The first is the BARF-1 gene, which is frequently expressed in the type I latent infection and reported to have a unique transcription pattern in EBVaGC.[39] The BARF-1 gene is able to immortalize primary monkey and human epithelial cells *in vitro*,[40,41] and transfection of BARF-1 into the rodent fibroblast cell line BALB/c 3T3 or into the EBV-negative B cell line, Louckes, resulted in tumorigenic transformation.[42,43] The second candidate is EBER-1 and/or -2 gene, which are by far the most abundant viral transcripts in latently EBV-infected cells and confer colony formation in soft agarose, tumorigenicity in immunodeficient mice, and resistance to apoptosis in Burkitt lymphoma cells.[44] Interestingly, recently it has been found that EBER-1 induce the secretion of IGF-I as an autocrine growth factor in EBVaGC,[45] and sequences containing a consensus ATF site upstream of the EBER-1 gene are important for EBER-1 expression.[46] The third candidate is the LMP2A gene. Comparison between EBVaGC and healthy donors have found that strains detected in EBVaGC tend to have LMP2A gene with threonin substitution at codon 348, which corresponds to HLA A-11 restricted CTL epitope.[47] This substitution may confer an advantage for viral persistence in tumor cells. Thus, polymorphisms or differences in expression of BARF-1, EBER's or LMP2A gene(s) might be associated with specific genotype that contribute to the EBVaGC carcinogenetic process.

EBV type 1 was identified in most of EBVaGC and healthy donors. The predominance of this strain is in agreement with its worldwide distribution.[18,19,36,38,44,48] The prototype F at BamHI-F region was the most common finding in our healthy donors as well as EBVaGC, a finding also in agreement with previous studies in Asian countries.[24,25] In this study, we found 2 recombinant strains among healthy donors (type I/XhoI + and type "i"/XhoI −). These findings might be explained by total or partial recombination during replication of the 2 wild-type EBVs (type I/XhoI − and type "i"/XhoI +).[49] Since type I/XhoI − is mainly from Asian origin and type "i"/XhoI + is common in Western countries, our findings correlate with ethnic distributions in our study area.[50] Thus, the generation of these new EBV recombinants might be due to the presence and mixing of different ethnic populations infected with EBV in this region. In this context, we cannot rule out the presence of more than 1 strain in healthy donors, since it has been described in up to 23% of normal individuals.[51,52] However, our PCR assay was not designed for detecting multiple strains and probably underestimates a more complex spectrum of EBV strains present in the throat of some healthy control individuals.

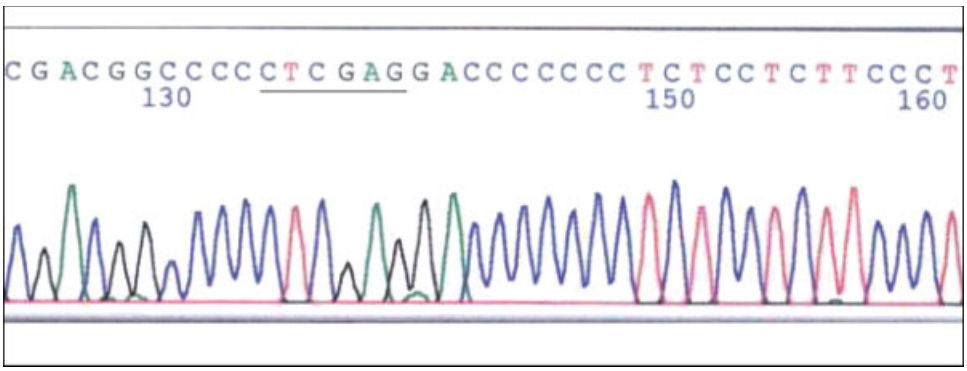

FIGURE 4.

In summary, the present study showed that the most of EBVaGC patients harbor a distinctive EBV strain (type "i"/XhoI +), but in healthy donors, this strain was as common as other 3 strains. In addition, although in a small subset of cases, the virus harbored in the orophaynx in cases of EBVaGC is the same to the virus seen in the stomach. Our findings identified a healthy population group that share the same strain that predominate in EBVaGC cases. It could be of interest to carry a extensive cohort studies following these individuals longitudinally to evaluate the risk to develop EBVaGC.

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