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Short Communication

Advances in the investigation of the Epstein Barr Virus (EBV) in Colombia during the last 20 years

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Introduction

Epstein Barr Virus (EBV) belongs to the family *Herperviridae*, subfamily *Gammaherpesvirinae*, genus Lymphocriptovirus [1]. It is one of the most common human viruses as most people get infected with EBV at some point in their lives. EBV spreads most commonly through bodily fluids, primarily saliva. Although viral DNA has been detected in breast milk and genital secretions [2], the evidence for sexual transmission is extremely limited [3].

EBV consists of a 172 Kpb double-stranded linear DNA, which is protected by a 100 nm diameter icosahedral symmetry capsid and composed of 162 capsomeres that together make up the viral nucleocapsid. The nucleocapsid is surrounded by a protein matrix, arranged asymmetrically, called the integument [4]. EBV is generally found in the episomal form within cells although in some cases has been described random integration of viral DNA into the host genome [5]. Even though EBV potentially codes for more than 85 proteins, only a few are currently well known: six nuclear antigens (EBNAs 1, 2, 3A, 3B, and 3C, and EBNA-LP); three latent membrane proteins (LMP) 1, 2A, 2B, also known as latent genes; small non-polyadenylated RNAs, EBER 1 and 2; microRNAs (miR-BHRF1 and miR-BART); and several early lytic genes [6].

The virus has shown to be involved in the etiopathogenesis of tumors such as nasopharyngeal carcinoma, Burkitt lymphoma (BL), and possibly in some cases of Hodgkin lymphoma (HL). Furthermore, EBV is the main causative agent of acute infectious mononucleosis (IM), a common syndrome characterized by fever, sore throat, extreme fatigue, and swollen lymph glands. In adolescents, EBV is responsible for the 35 to 70% of IM cases and patients diagnosed with HL have shown to have an increased risk of developing this disease if they had previously presented IM [7-9].

Apart from the oral cavity, EBV DNA has been detected in a great variety of tissues. This, in addition to its high geographic heterogeneity, has led to suspect the existence of different subtypes or viral variants [9]. Some variants are described according to their geographical origin: Med+ and Med- from the Mediterranean region; China1 and 2, from China; NC, from the North Carolina region; and Alaskan, from the Alaska region. Other variants are identified in pathogenic cell lines: Raji, AG876, and MUTU, from African patients with BL [10]; the GD1, GD2, Cao, Med81, and AKATA strains, from patients with nasopharyngeal carcinoma (NPC) or BL [11]; and B95.8, from a patient with IM [12]. These variants can be widely distributed, although at low frequencies in different parts of the world, which has caused that currently there is no clear consensus about the EBV classification [13].

Thus, appropriate virus detection and classification methods are subjects of study under development. Progress in these areas will allow for timely detection and will prevent disease development. In Colombia, EBV studies were traditionally focused on the relationship between the virus and the disease development. However, new studies have expanded the knowledge about the presence of pathogenic variants that show a genotype-phenotype association. Accordingly, an up-to-date revision of these studies is presented in the following paragraphs.

Methods

Literature research was performed in the PubMed and Google Scholar databases. PubMed's MeSH terms IM, Epstein-Barr virus, human herpesvirus 4, HHV-4, mononucleosis, lymphoma, lymphoproliferative disorder/cancer, multiple sclerosis, variants, phylogeny and Colombia, were used.

Then, title and abstract screenings were made to assess whether the search results were of relevance for the aim of this short communication. Systematic reviews, case reports, comparative studies and meta-analyses that included Colombian patients were prioritized.

All investigations that presented detection of EBV in symptomatic or asymptomatic Colombian patients were considered. Contradictory results and alternative perspectives on the issue were noted and presented.

Results

A total of 10 scientific articles were found published from 1999 to 2020 in national and international journals that included patients affected with EBV in Colombia. The results and alternative perspectives obtained from these publications will allow us to focus future research efforts on the genotype-phenotype association (Table 1).

A summary of the publications carried out so far

1999: Carrascal et al. reported 99 consecutive cases of gastric adenocarcinoma using *in situ* hybridization for EBV-encoded small non-polyadenylated RNA-1 (EBER-1). The study looks for EBV in malignant epithelial cells of 66 men (mean age: 62 years), and 33 women (mean age: 60 years). The investigators found 46 gastric adenocarcinomas of the intestinal type and 53 of the diffuse type, without reporting any cases of lymphoepithelioma. Ten of the cases were positive for EBV (10.1%), 9 men and 1 woman. Out of the positive cases, 5 were of the intestinal type and 5 of the diffuse type. In 26 cases, the location of the tumor could not be known from the available registries. Of these, 3 were positive for the EBV. Among the remaining 73 cases, one case originated in the post gastrectomy gastric remnant. No lymphoepithelioma was found in this series, which it deserves further studies to establish the frequency of this morphology in Colombia, both in the stomach and elsewhere- It was concluded that EBV is etiologically associated with at least 10% among all gastric adenocarcinomas [13].

2003: Carrascal et al. estimated the incidence of EBV-associated gastric carcinoma and provided clinic-pathological characteristics for 178 consecutive cases of gastric carcinoma, diagnosed from 1996 to 1998 [14]. The study was carried out at the Hospital Universitario del Valle in Cali, Colombia. The mean age of the cases was 60 years in men and 58 years in women. Using the EBV-encoded small RNA-1, detected by *in situ* hybridization assay in samples of paraffin-embedded tissue, the researchers identified 23 cases of EBV-GC (13%). The gender and age-specific incidence of EBV-GC was found to be 4.1 and 1.4 for men and women, respectively, after adjustment of age using the world standard population. The EBV-GC represented the 33% (8/24) of the carcinomas located in the cardia of the stomach, 14% (6/43) of the carcinomas in the middle part of the stomach, and 7% (6/81) of carcinomas in the antrum. Specific histology analysis using the Lauren classification revealed that the EBV-GC ratio was not different between diffuse and intestinal type carcinomas (13% in both types). The frequency of EBV-GC was slightly higher in advanced tumors, which involved serosa. Further analysis of clinic-pathological features of EBV-GC using a larger number of cases would give invaluable insights into its etiology. [14].

2004: Quijano et al. determined the presence of Epstein-Barr virus in 67 lymph nodes of patients with a confirmed diagnosis of HL by *in situ* hybridization for the detection of EBV RNA transcripts, and by immunohistochemistry for the detection of the oncogenic protein LMP-1. They identified a 56.7% relationship between EBV and HL by LMP1 analysis. The presence of the virus by histological type was 69.81% in nodular sclerosis, 85.71% in mixed cellularity, and 40% for lymphocyte rich. Epstein-Barr virus was detected more frequently in children (84.2%) compared to adults (60.4%). The results showed that the analyzed Hodgkin lymphoma cases can be classified in an intermediate epidemiological pattern, based on histological subtypes, age groups and the presence of Epstein-Barr virus in tumor cells. These results suggest that the presence of Epstein-Barr virus in all new cases of Hodgkin lymphoma may have

Source	Article Type	N	Technique used
Carrascal et al. 1999 [13]	Clinical study	99	*in situ* hybridization for EBV
Carrascal et al. 2003 [14]	Comparative Study	178	*in situ* hybridization for EBV
Quijano et al. 2004 [15]	Original article	67	*in situ* hybridization for EBV
Campos et al. 2006 [16]	Research Communication	368	ISH assay of paraffin-embedded tissue samples, interview
Mesa et al. 2015 [17]	Case series	7	review of medical records
Nieto-rios et al. 2016 [18]	Retrospective study	8	Review of medical records
Bohorquez et al. 2016 [19]	Original article	17	Conventional PCR
Amaya et al. 2019 [20]	Observational Study	55	Review clinical and pathological records
Giraldo-Ocampo et al. 2019 [21]	Original article	374	Conventional PCR, Real Time PCR
Arturo-Terranova et al. 2020 [22]	Original article	84	Conventional PCR, phylogeny

Table 1: Summary of studies on EBV in Colombia from 1999 to 2020.

important implications as a possible prognostic factor for treatment response [15].

2006: Campos et al conducted a case-control study in Cali, Colombia with 368 newly diagnosed gastric carcinoma patients from September 2000 to June 2003, including 42 cases of EBV-associated gastric carcinoma (EBV-GC). Information about lifestyles, eating habits and occupational exposure was obtained through a questionnaire. The frequency of EBV-GC was related to the birth order (P for trend=0.025), being less frequent among eldest sons and daughters (P=0.007). The decreased EBV-GC risk among eldest children, suggests that first EBV infection at older ages is related to a decreased EBV-GC risk. The observations also suggest that most of the Latin American populations, including Colombia, may get the first EBV infection at ages older than the Japanese population because of different cultural practices such as sharing bedrooms, although they do not have data of seroprevalence among Colombian children [16].

2015: Mesa et al. described findings related to use of the viral load for the Epstein-Barr virus for follow-up of pediatric patients undergoing liver transplantation in a fourth-level institution in Medellín, Colombia. They reviewed 7 clinical histories of liver transplant patients and recorded demographic data, the reason for transplantation, the timing of the appearance of Epstein-Barr virus-positive viral load, the evolution of the viral load, the effects of immunosuppressive and antiviral drugs, and the outcome of the patients were registered. The average age of the patients at the time of transplantation was 28.4 months. Antiviral drugs such as ganciclovir, valganciclovir, and immunoglobulin against cytomegalovirus, did not seem to exert an adequate and sustained decrease in viral load. They concluded that the degree of immunosuppression seems to be the main factor for controlling the replication of the Epstein-Barr virus [17].

2016: Nieto-Rios et al. conducted a retrospective study that included patients with a diagnosis of lymphoproliferative disease after kidney transplantation between January 2011 and July 2014. The diagnosis was made by histopathological analysis of the lesions. *In situ* hybridization in biopsies was performed to determine the presence of Epstein Barr virus, and by immunohistochemistry, to detect the presence of LMP1. The authors included eight cases, with variable clinical presentations, most of them corresponded to monomorphic histology. Association between EBV expression and immunohistochemical staining was confirmed in 85% of the patients. Viral load measurement for EBV at diagnosis was negative for 3 patients (37.5%). Twenty-five patients (25%) had renal graft tumor involvement and 12.5% primary lymphoma of the central nervous system. All patients were managed with reduced immunosuppression, conversion to m-TOR (except one who lost the graft at diagnosis), and rituximab-based treatment. The overall response rate was 87.5% (62.5% complete response, and 25% partial response). Survival was 87.5% with a median follow-up of 34 months. Despite the low numbers of cases included in the study, some conclusions are worth mentioning. For example, it can be of relevance that among several risk factors for lymphoproliferative disorders, EBV may be an important one, since was found in 60-70% of the studied cases [18].

Bohorquez et al. evaluated the behavior of the viral discharge of herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), human cytomegalovirus (CMV), and Epstein-Barr virus (EBV) using conventional PCR, in the saliva of 17 pediatric transplant patients of hematopoietic precursors associated with low leukocyte counts and allogeneic transplantation in the Transplant Unit of the HOMI Foundation at the Hospital de la Misericordia, Colombia. They detected HSV-2 and CMV DNA in the saliva of four patients, and EBV DNA was detected in nine patients with leukopenia. In contrast, they did not detect HSV-1 DNA in saliva. Additionally, four out of 17 patients showed a simultaneous shedding of CMV and EBV. These four patients who had CMV deletions also had EBV, supporting the hypothesis that CMV discharges are more likely to be detected on days when EBV is also detected. CMV discharge in saliva was found to be independent of reactivation of infection in peripheral blood mononuclear cells, as has been concluded in other studies. In the evaluated patients, it was observed that the discharge of the herpes virus in saliva was not constant or permanent and that its behavior is different in peripheral blood. As a conclusion, they were able to corroborate that saliva can be used as a safe and easily obtained mean to evaluate asymptomatic viral discharges [19].

2019: Amaya et al. carried out an observational descriptive study of a historical cohort where they reviewed the clinical and pathological anatomy records of patients diagnosed with diffuse large B-cell lymphoma (LDCBG). They also performed *in situ* hybridization to detect EBV (EBER). Between 2011 and 2017, they reviewed the medical history of 55 patients diagnosed with LDCBG. Sixteen (16) % of the included cases were EBV positive and had tumors associated with the non-germinal center subtype (89%). Studied cases had a nodal presentation (56%) and were more prevalent in men (68%), had a younger age of presentation (median 48 years). Lethality was observed in 56% of cases. Patients with LDCBG and positive EBV presented the non-germinal subtype more frequently. This presentation was more prevalent in younger patients and was associated with a worse prognosis. The EBER is not a routine test, but they recommended its inclusion in the pathology package for DLBCL patients [20].

Giraldo-Ocampo et al. carried out a retrospective cross-sectional study where 374 saliva samples, taken between 2015 and 2016, were analyzed using conventional PCR and Real-time PCR for EBV detection. The association between the detection of viral DNA and demographic characteristics was evaluated by an opportunity ratio analysis to assess the association measure. Viral DNA was detected in 45% (167/374) of the oral samples. The detection rate of EBV was 26% (96/374). With the real-time PCR technique, viral DNA was detected in 71 samples that gave a negative result with conventional PCR (167/374). The Kappa coefficient in the concordance analysis of the results for the techniques was equal to 0.42. The higher viral presence was found in school children in the eighth and ninth grades (p=0.004); where the 14-year-old students presented a 2.4 times greater risk for virus detection (95% CI: 1.12-4.9). Men presented a greater number of positive cases compared to women; however, these differences were not statistically significant (p=0.603). This study evidenced the exposure of EBV in the oral cavity of high school students and suggested the need for the generation of surveillance actions to monitor the implications of these findings on the health of schoolchildren [21].

2020: Arturo-Terranova et al. identified and characterized the EBV variants detected in the oral cavity of 84 adolescents from Cali, Colombia, using conventional PCR, purification and sequencing of the EBNA3C gene to subtype the virus and the C-ter domain of

the LMP1 protein. Besides, a phylogenetic and nucleotide variant analysis of the obtained sequences was performed. The authors compared their findings to pathogenic and geographic variants reported in the GenBank. It was found that the predominant viral subtype was EBV-1 (79%); 72.6% of studies cases were grouped with the pathogenic variant Raji, derived from B lymphocytes from a patient with Burkitt lymphoma; 13.7% were related to a variant of geographical origin in the Mediterranean; 13.7% of subjects were not grouped with any of studied reference variants. The phylogenetic reconstruction of LMP1 variants showed that none of the EBV detected in Cali was grouped with the prototype pathogenic variant B95.8, predominant in the European population. Furthermore, it was observed that the geographic variants China 1, GD2, Cao and Akata are closely related to each other, as are the two geographical variants of the Mediterranean subtype, while the geographical variants China 2, NC and Alaskan form independent groups from each other and with respect to the other variants. This was the first Colombian study where variants associated with EBV were reported in the country. However, it was emphasized that new studies are required to characterize unidentified variants and determine their pathogenicity and geographic prevalence [22].

Conclusions

Due to the high prevalence of EBV-infected individuals throughout the world and the complications in the pathologies associated with the virus, mainly those related to oncological effects, preventive measures should be prioritized, ideally for early diagnosis. Also, future research should focus on the causalities and associations to contribute to the understanding of the pathogenic mechanisms of EBV.

The studies carried out in Colombia regarding the clinical association of EBV have been mainly based on its association with gastric cancer and HL and allow to understand part of the pathologies associated with the viral disease. The studies based on the phylogenetics of the virus and its association with pathogenic or benign variants have opened the doors to new research associated with the relationship between the viral variants and the disease. It is still necessary to clarify whether these variations are ethnic or geographically correlated, or if they are disease-related, thus being important to characterize the epidemiologic genetic distribution of EBV strains in these populations.

Currently, there is no evidence to implement specific prophylactic measures to prevent EBV infection. However, it is recommended to avoid exposure by daily hygiene practices such as hand washing, avoid sharing utensils and, for immunosuppressed patients or transplant candidates, avoid contact with potentially infected secretions (respiratory or saliva).

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Conflicts of Interest

The author declares no conflict of interest.

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