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ARTICLE 1

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PREVALENCE OF EPSTEIN BARR VIRUS IN TISSUES WITH SUSPECTED LYMPHOMA OF LYMPHOMA RECEIVED BY THE DEPARTMENT OF CLINICAL LABORATORIES AND PATHOLOGY AT ROOSEVELT HOSPITAL BETWEEN 2022 AND 2024, USING THE EBER-ISH MOLECULAR TEST

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Abstract: Epstein-Barr virus (EBV) is associated with various lymphomas and lymphoid neoplasms, so its detection in tissues suspected of malignancy is essential for diagnosis and understanding its role in oncogenesis. In Guatemala, evidence of EBV presence in tissues studied by pathology remains limited. The objective was to determine the prevalence of EBV using the EBER-ISH molecular biomarker in tissues with clinical suspicion of lymphoma received at Roosevelt Hospital between 2022 and 2024, and to describe its distribution according to sociodemographic and histological characteristics. **Methodology**: Descriptive cross-sectional study with 100 tissue samples with clinical suspicion of lymphoma, processed by in situ hybridization for EBER-ISH. Sociodemographic variables, histological classification, and biomarker positivity were analyzed. Descriptive statistics and Fisher's exact test were applied to evaluate the association between histology and positivity. **Results**: The prevalence of EBV positivity was 48.0% (95% CI: 37.9–58.2). Positivity was concentrated in malignant tissues (47.0%). The mean age was 45.7 years; 51% were men. Among those who tested positive, the most frequent diagnoses were Hodgkin lymphoma e lymphoma (41.7%), NK cell lymphoma (25.0%), and diffuse large B-cell lymphoma (10.4%). **Conclusion**: A high prevalence of EBV was observed in tissues with clinical suspicion of lymphoma, highlighting its greater presence in malignant subtypes, especially Hodgkin lymphoma. These findings reinforce the clinical relevance of EBV in lymphoid neoplasms and underscore the need for studies evaluating its prognostic role and potential usefulness as a therapeutic biomarker.

Keywords: Epstein-Barr, EBER-ISH, Lymphoma, Prevalence, Histopathology

INTRODUCTION

Epstein-Barr virus (EBV) is an infectious agent that has tropism for lymphoid cells and occasionally for epithelial cells. EBV is found in saliva and is therefore transmitted by coughing, sharing food, or kissing. Low amounts of EBV can be detected in the saliva throughout the lives of infected individuals without causing acute or chronic disease (Medina-Ortega, 2017; Fugl, 2019).

When EBV infects B cells, the virus can limit its gene expression to only nine proteins, which prevents recognition by cytotoxic T lymphocytes, resulting in latent infection. Different patterns of latency (I, II, III) have been described, expressed and associated with different EBV-associated lymphoproliferative conditions, such as Burkitt's lymphoma (BL), Hodgkin's lymphoma (HL), and HIV-associated lymphomas, respectively (Damania, 2022; Toro Montoya, 2023).

Globally, the prevalence of EBV in developing countries is 90%, occurring at an early age, while in developed countries, primary EBV infection occurs in 50% of cases, occurring in adolescents and adults. The prevalence of EBV varies mainly according to sociodemographic characteristics and cultural changes between countries (Medina-Ortega, 2017).

Latin American countries report different EBV seroprevalence rates, depending on sociodemographic characteristics and cultural changes between countries. In Chile, the seroprevalence of EBV is 76.7%,

and in Brazil, it is 80% (Giraldo-Ocampo, 2019). Donzel, meanwhile, described the frequency of positive cases of HL associated with EBV in Latin America, with proportions ranging from 50% to 74% (Donzel, 2022).

Studies in Guatemala have revealed a prevalence of 21.1% of EBV in biopsies of gastric cancer patients diagnosed by in situ hybridization of *Epstein Barr encoded RNAs* (EBER) (Lange, 2020). For his part, Guox documented in his thesis in 2018 a prevalence of HL of 8.4% (Guox, 2018).

The diagnosis of EBV infection in tissue samples is important due to the associated pathologies that have been described, especially lymphomas; the type of treatment reduces morbidity and mortality in patients with malignant neoplasms associated with EBV infection. There are several diagnostic methods for tissue, but the gold standard is molecular testing, in situ hybridization of Epstein Barr encoded RNAs (EBER-ISH) (AbuSalah, 2020).

Immunohistochemistry is a first-line test used for the diagnosis of EBV-associated lymphomas. It has high sensitivity for these diagnoses due to its ability to detect EBV-infected memory cells. In situ hybridization is used as a diagnostic method for EBV in tissues, following the issuance of the histopathological diagnosis; in situ hybridization is the standard method for the diagnosis of EBV in tumor cells. This method is capable of detecting different EBV mutations in tissues such as microRNAs (EBER), latent membrane proteins (LMP1 and LMP2), and nuclear antigens (EBNA) (Karaarslan, 2015; Gulley, 2002; Hassan, 2006).

The molecular technique of in situ hybridization, EBER-ISH, has greater specificity in terms of cellular localization and high sensitivity due to the detection of the expression of certain molecules; it also has greater sensitivity in identifying EBV in paraffin tissue samples, especially in cases of non-Hodgkin lymphoma (NHL). Both tests, immunohistochemistry and in situ hybridization, show high concordance (95%), with immunohistochemistry being defined as the recommended test for the diagnosis of EBV-associated lymphomas and in situ hybridization as a confirmatory test. A review of the literature shows that better results are obtained when the tests are used concomitantly (Hassan, 2006; Medina-Ortega, 2017).

In Guatemala, there is insufficient information on tests for the identification of EBV in tissue analysis. Due to the lack of immunohistochemical and molecular diagnosis, many patients die without having started their cancer treatment as a result of an unestablished diagnosis (Guox, 2018).

The main objective of this research was to determine the prevalence of EBV through molecular testing, EBER-ISH, in tissues with clinical suspicion of lymphoma, from samples received at the Department of Clinical Laboratories and Pathology, Roosevelt Hospital, during the years 2022 to 2024. In addition, we sought to determine the percentage of test positivity, establish the association between the general classification and the EBER-ISH result, and categorize the histological type with a positive EBER-ISH result.

METHODOLOGY

Study design

Descriptive, cross-sectional study.

Population

Biopsies received and processed by the Department of Clinical Laboratories and Pathology, Roosevelt Hospital, from patients with clinical suspicion of lymphoma.

Sample

Biopsies received and processed by the Department of Clinical Laboratories and Pathology, Roosevelt Hospital, on which the EBER-ISH biomarker was performed to detect EBV in tissues with clinical suspicion of lymphoma.

Sampling design

The non-probabilistic method was used during the corresponding period from 2022 to 2024.

Inclusion criteria

Samples from any anatomical site studied (lymph node and other anatomical sites); from both sexes; aged 16 years or older, from patients with clinically suspected lymphoma.

Exclusion criteria

Tissues without fixation medium or inadequate fixation, slides with inadequate staining of the EBER-ISH biomarker, inadequate control of the EBER-ISH biomarker.

Data collection instruments

A Microsoft Office Excel 2013 spreadsheet was used to transcribe the variables of interest for the creation of a data matrix.

Data collection procedure

A data matrix was created, transcribing the variables originally recorded in the computer system: sex, age, general histological classification (benign/malignant), histological type of neoplasm (ALL-B, LB, LH, NHL, LDCBG, plasmablastic lymphoma, ALL-T, NLL, lymphoepithelial carcinoma, other neoplasms), EBER-ISH biomarker result (positive/negative), and final diagnosis (e.g., 1 LB EBER-ISH amplified/positive) (e.g., 2 Reactive lymphoid hyperplasia associated with EBV infection/not associated with EBV infection).

Statistical analysis (data management)

All variables included in this study: sex, age, general histological classification, histological type of neoplasm, EBER-ISH biomarker result, final diagnosis ; were collected from the Surgery Program of the Pathology section and standardized in a database sheet in Microsoft Office Excel 2013; they were then analyzed using Jamovi 2.3.28 statistical software. The data recorded in this database were all those that met the selection criteria.

- Epidemiological characteristics, age, and sex were described; a description of the qualitative identification variables was made using absolute frequencies and percentages. For quantitative variables, the mean was used, as appropriate.
- The results of the EBER-ISH biomarker in tissues were described using frequencies and percentages.
- The Chi-square test or Fisher's exact test was applied, as appropriate, to establish the association between the general histological

classification and the result of the EBER-ISH biomarker in tissues of patients who attended Roosevelt Hospital from January to December 2022 to 2024.

- The histological type of neoplasm with a positive result for the EBER-ISH biomarker in tissues was categorized by frequency and proportion in patients who attended Roosevelt Hospital between January and December 2022 and 2024.

Resources

Institutional resources

Surgical Program, Pathology Section, Department of Clinical Laboratories and Pathology, Roosevelt Hospital.

Human Resources

- Research team:
  - Researcher, pathologist, Department of Clinical Laboratories and Pathology, Roosevelt Hospital.
  - Advisor, pathologist, Department of Clinical Laboratories and Pathology, Roosevelt Hospital.
  - Internal reviewer, biological chemist from the Dr. Carlos Mejía Villatoro Comprehensive Care Unit for HIV and Chronic Infections at Roosevelt Hospital.
- Medical collaborators, who will evaluate patients and take tissue samples when lymphoma is clinically suspected.
- Collaborators from the Tissue Processing Laboratory and Immunohistochemistry Laboratory, Department of Clinical Laboratories and Pathology, Roosevelt Hospital: four laboratory technicians performed tissue processing and two laboratory technicians performed the EBER--ISH biomarker.
- Heads of the Anatomical Pathology Area, Department of Clinical Laboratories and Pathology: chief physicians evaluated the EBER-ISH biomarker results and issued the final results.

Materials

- **Molecular testing by in situ hybridization biomarker (EBER-ISH):**

EBER-ISH biomarker result: The EBER-ISH was performed according to the manufacturer's instructions, Ventana, ROCHE, with the respective internal work protocol, in the Immunohistochemistry Laboratory of the Department of Clinical Laboratories and Pathology at Roosevelt Hospital.

Procedures

- **EBER-ISH biomarker:** The procedure was performed following the guidelines indicated by the manufacturer and internal work protocols; it was carried out in the Immunohistochemistry Laboratory of the Department of Clinical Laboratories and Pathology at Roosevelt Hospital.

Ethical considerations

This research did not require informed consent from patients, as the unit of analysis was the results recorded in the information system (Surgical Program) on a routine basis, which were delivered to the different departments of Roosevelt Hospital and/or to patients for the respective clinical approach. No use was made of names, medical records, or unique patient identification codes, thus ensuring patient confidentiality. In addition, authorization was obtained from the authorities of the Department of Clinical Laboratories and Pathology at Roosevelt Hospital and the Department of Teaching and Research at the same hospital to carry out this research. Approval recorded in minutes No. 808, point No. 4, dated October 27, 2025.

RESULTS

This study analyzed the sociodemographic characteristics and histological findings of samples with clinical suspicion of lymphoma, as well as the expression of the EBER-ISH biomarker for the detection of Epstein-Barr virus (EBV). The results obtained allow us to describe the distribution of the variables included and establish the possible association between biomarker positivity and the different histological classifications observed in the tissues processed during the period 2022 to 2024.

Of the 100 participants, 47% were women (95% CI: 36.9–57.2) and 51% were men (95% CI: 40.8–61.1). Age was normally distributed (Shapiro–Wilk test r W = 0.976; p = 0.064). The mean age was 45.7 years (95% CI: 42.2–49.3), with a standard deviation of 18 years.

Table 1 shows the distribution of EBER-ISH test results in the study population. It was observed that 48.0% (95% CI: 37.9–58.2) of the samples were positive for the biomarker, showing a similar frequency to the negative results (52.0%).

Table 2 shows the relationship between the overall histological classification and EBER-ISH biomarker positivity. Biomarker positivity was concentrated in malignant cases (47.0%), while benign cases were predominantly negative (5.0%), with only one positive case (1.0%). Although the highest frequency of positivity was recorded in malignant tissues, Fisher's exact test (p = 0.207) did not show a statistically significant association between the two variables.

Table 3 shows the distribution of histological types that tested positive for the EBER-ISH biomarker. Hodgkin lymphoma was the most common diagnosis among positive cases (41.7%), followed by NK cell lymphoma (25.0%). The other histological subtypes, including diffuse large B-cell lymphoma, T-cell lymphoma, and other neoplasms associated with EBV infection, had frequencies less than or equal to 10.4%.

DISCUSSION

The EBER-ISH molecular test is currently the gold standard for the association of EBV in lymphomas and EBV-associated neoplasms. *EBER-ISH* is considered the *gold standard* for detecting EBV-associated neoplasms in tissue samples, with 100% sensitivity and the advantage of detecting EBV infection regardless of the latency pattern expressed in the tissue. Although EBER-ISH is the gold standard for detecting EBV-associated malignancies, molecular determination of EBV viral DNA, RNA,

EBER-ISH	Frequency	Proportion	95% CI	
			Lower	Upper
Negative	52	52.0%	41.8	62.1
Positive	48	48.0	37.9	58.2

Table 1. Distribution of EBER-ISH test results in the study population (n = 100)

Classification	EBER-ISH				Total	
	Negative		Positive			
	f	%	f	%	f	%
Benign	5	5.0%	1	1.0%	6	6.0%
Malignant	47	47.0%	47	47.0%	94	94.0%
Total	52	52.0%	48	48.0%	100	100.0%

Fisher's exact test, $p = 0.207$

Table 2. Association between overall histological classification and EBER-ISH positivity (n = 100)

Histological diagnosis	Frequency	Proportion
EBV-associated squamous cell carcinoma	1	2.1
Lymphoepithelial carcinoma associated with EBV infection	3	6.3
Reactive follicular hyperplasia associated with EBV infection	1	2.1
LB associated with EBV infection	1	2.1
LCT associated with EBV infection	4	8.3
LDCBG associated with EBV infection	5	10.4
LH associated with EBV infection	20	41.7
LNK associated with EBV infection	12	25.0
Plasmablastic lymphoma associated with EBV infection	1	2.1

Table 3. Distribution of EBER-ISH positive histological types (n = 48)

and viral load is widely used in the clinical evaluation of EBV-associated tumors (Abu-Salah, 2020). Other diagnostic methods, such as polymerase chain reaction (PCR), have a 95% concordance with the in situ hybridization method; however, PCR has a sensitivity of 96% in detecting the EBV genome in tissue samples (Hassan, 2006).

In Guatemala, immunohistochemistry or in situ hybridization is used to diagnose EBV-associated pathologies in paraffin-embedded tissue samples. Loughrey mentions that the EBER-ISH method has unequivocal results, making it a very useful auxiliary diagnostic tool for the diagnosis of lymphomas in the appropriate clinicopathological context (Loughrey, 2004).

The date on which the EBER-ISH technique began to be used in the field of pathology in Guatemala at the private level is unknown. However, the pioneering group in the use of this methodology in the public system was the Department of Clinical Laboratories and Pathology at Roosevelt Hospital, with this research in November 2022, using the Ventana BenchMark ISH methodology from Roche.

The Ventana probe, Roche, is designed for use in the qualitative detection of EBV EBER RNA in paraffin-embedded, formalin-fixed samples by in situ hybridization. A positive reaction for EBV EBER RNA in target cells is indicated by a clearly magenta-stained nucleus in relation to the background cellularity (Roche, 2024; Roche, 2025).

The study by Donzel (2022), based on the Lymphopath electronic clinical medicine network, included 756 cases of lymphoma; 110 were HL (14.6% of the total analyzed), 58% male with a mean age of 38 years (interquartile range 29-61); and 575 were B-cell lymphomas (BCL) (76.1%), 57% of whom were male with a mean age of 71 years (interquartile range 60-78), while in 71 NK cell lymphomas (NCL) (9.4%), 73% were in men and the mean age was 67 (49-77). These data are similar to those found in the present study, in which, of the 100 participants, 51% were men (95% CI: 40.8–61.1) and 47% were women (95% CI: 36.9–57.2); and in which age was normally distributed (Shapiro–Wilk test W = 0.976; p = 0.064), with a mean of 45.7 years (95% CI: 42.2–49.3) and a standard deviation of 18 years.

Regarding the relevance of the age distribution of EBV-associated HL, EBV-positive cases are more common in *children and adults over 80 years of age.* It is suggested that HL arising in children and young adults may be a consequence of primary EBV infection, while the peak in older adults may be attributed at least in part to senescence of EBV immunity and an increasing EBV burden (Shannon-Lowe, 2017).

In another study in Fujian, led by Wang (2021), the study was divided into four age groups: 0-14, 15-34, 35-49, and 50 years and older. Of the 134 patients studied, 83 were men (61.9%) and 51 were women (38.1%); the median age at diagnosis was 31 years; most were young patients between 15 and 49 years of age. The EBV positivity rate, as determined by the EBER-ISH test, in patients aged 0 to 14, 15 to 34, 35 to 49, and 50 and older was 75.0%, 31.9%, 55.6%, and 63.3%, respectively (p = 0.004); in patients aged 15 to 74 years, the EBV positivity rate increased gradually with age. This contrasts with the present study, in which the majority were men (51%) and age showed a normal distribution (p = 0.064).

In the study led by Kuri in the United Kingdom, a seroepidemiological investigation was conducted with serum samples from 2,325 people aged 0 to 25 years to assess the prevalence of detectable EBV antibodies; it was determined that 85.3% of the individuals were EBV seropositive. Kuri then determined that the prevalence varies with age. Positivity increases with age, affecting more than 95% of the adult population worldwide (Kuri, 2020).

Hoover mentions that the prevalence of primary EBV infection is higher in children and adolescents with larger households, lower family income, and lower parental education levels. Prevalence is higher at younger ages because there is greater interaction at these ages, with a higher risk of contact and sharing of utensils. By adulthood, about 95% of the population has been exposed to EBV. Primary infection often occurs in young children or adolescents and is transmitted through close contact with saliva or bodily fluids, with prevalence increasing with age to affect more than 95% of the adult population (Hoover, 2023).

The cumulative EBV infection suggests that EBV is a determining factor in the pathogenesis of EBV-associated pathologies such as multiple sclerosis and lymphomas, acting through mechanisms such as molecular mimicry and immune response regulation. EBV infection is the factor that most increases the risk of developing EBV-associated lymphomas, and determining genetic factors is also fundamental in the development of lymphomas (Hernández, 2025).

Immunity is essential for controlling EBV, which, in turn, has developed sophisticated strategies to evade it and establish latent and persistent infections in B cells. The complex interaction between the two determines the outcome of the infection: in healthy individuals, the immune response keeps the virus under control, while in immunodeficiency, EBV can cause serious diseases such as lymphomas due to the uncontrolled proliferation of infected cells (Hatton, 2014). Shannon-Lowe states that the clinical presentation of EBV infection in older adults can be attributed to the senescence of EBV immunity and an increasing EBV burden (Shannon-Lowe, 2017).

In the study led by Patel (2022), which reviewed the PubMed database of cases published between 1977 and 2022 and focused on the three most common malignancies caused by and associated with EBV: HL, BL, and SNLEC, with the aim of highlighting the frequency with which EBV positivity is detected. Patel's study emphasized determining which EBV markers were most indicative of prognosis and the likelihood of developing EBV-associated lymphomas.

After incorporating the EBV genome into the cell nucleus, EBV can begin its lytic phase or enter a latent phase. EBV converts the host's resting B lymphocytes into lymphoblastoid cell lines with latent infection. The latent phase is classified into four types (0, I, II, and III); EBV establishes latency in B lymphocytes through the expression of viral genes, such as EBNA, LMP, and small EBER RNAs, promoting cell proliferation and the immortalization of B lymphocytes, which leads to the malignant transformation of infected B lymphocytes. The different types of latency, defined by the pattern of gene expression, are associated with various types of lymphomas. For example, type I latency is associated with B-cell lymphoma (B-CL); type II latency is associated with diffuse large B-cell lymphoma (DLBCL); and type III latency is associated with lym-

phomas associated with HIV/AIDS (Silva, 2024; Ali, 2015; Medina-Ortega, 2017).

In Patel's study (2022), the aim was to determine, for HL, whether any relationship could be found between EBV status in HL tumors and the patient's overall prognosis. They analyzed a group of 134 untreated patients diagnosed with HL who tested positive for EBV by in situ hybridization. They found no association between EBV status and overall survival or failure-free survival, except in groups over 50 years of age, where EBV positivity indicated lower survival statistics. In contrast to the present study, in which 48% (48/100) of participants tested positive for EBER-ISH, no statistical association was found between overall histological classification and EBER-ISH positivity (p=0.207) (Table 1 and Table 2).

In the United States, the prevalence of EBV in adolescents was 82.9%, with minimal differences between the sexes. In England, the prevalence rate of EBV in young adults was 93% seropositive (Hoover, 2023). In the retrospective study conducted by Nilsson (2019) in Sweden, which included 48 cases of SNLEC, EBER-ISH positivity was determined in 75% of cases; that is, 36/48 patients had lesions with positive EBER-ISH expression. This contrasts with the present study, in which almost half of the patients tested positive for EBER-ISH (48.0%; 95% CI: 37.9–58.2) (Table 1); and of these, the most frequent histological type positive for EBER-ISH were patients diagnosed with HL (41.7%) and NKC (25%) (Table 3).

The proportion of EBV-positive HL cases varies among different populations; in developed countries, EBV-positive HL rates range from 20% to 50%; and it occurs mostly in older people and children. In contrast, rates of HL associated with EBV infection tend to be higher in low-income countries, occurring mainly in young people; possible confounding factors, such as low socioeconomic status, are taken into account. The association between HL and EBV infection is likely due to different levels of immunosuppression in the patient, both locally in the tumor tissue and systemically (Vrzalikova, 2021).

EBV is associated with 25% of NK and T-cell lymphomas, such as extranodal NK-cell lymphoma. In Donzel's study, there was a statistically significant association between EBER positivity and relapse ($p < 0.01$) (Donzel, 2022).

In Tellez's study (2014), 58 tissue samples were analyzed, diagnosed with EBV-associated lymphoid tissue disorders using a Lawrence Livermore microbiome detection array (LLMDA) and confirmed with the EBER-ISH test. These included 50 samples of malignant lymphoid tissue, of which 30 were B-cell lymphomas ( ), 2 were T-cell lymphomas (LCT), 2 were LNK, 6 were LH, and 10 were post-transplant lymphoproliferative disorders (PTLD). On the other hand, 8 were benign lymphoid tissue samples. Five of the 21 high-grade BCLs were positive for EBER-ISH, while all indolent BCLs were EBER-ISH negative; both NK/T-cell lymphomas were EBER-ISH positive, and one of the 6 HLs was positive for EBER-ISH. All benign tissues were EBER--ISH negative. Similar data were found in the present study, in which EBER-ISH positivity was concentrated in cases with malignant pathology, with 47% EBER-ISH positivity compared to 1% positivity in cases of benign pathology (Table 2).

The prognosis of EBV-associated HL is complex; in general, it depends on the age of the patient. EBV-positive HL in children and older adults is associated with lower survival; in contrast, in young adults, EBV--associated cases have been associated with better disease-free survival (Carbone, 2018).

The prognosis of EBV-associated NHL varies significantly depending on the stage of the disease; early and localized stages have a 5-year survival rate of 70% and can be successfully treated with radiotherapy. In contrast, advanced LNK disease has a poor prognosis, with treatments combining chemotherapy and autologous transplantation, but with lower survival rates (Takahara, 2021).

The findings in this study regarding the association between EBER-ISH positivity and overall histological classification, in which no statistical association was found (p = 0.207) (Table 2), are similar to those published in the study by Saikia (2016) conducted in India, in which no correlation was observed between EBV and age, sex, lymph node metastasis, stage, or histology. Saikia (2016) used chromogenic in situ hybridization (CISH) to determine EBV positivity in tissue biopsies from 51 patients diagnosed with nasopharyngeal carcinoma (SNLEC). Saikia's study found a statistically significant association between EBER expression and certain sociocultural variables, such as consumption of smoked foods (p = 0.02) and exposure to tobacco smoke (p = 0.02).

Donzel (2022) used the EBER-ISH test to determine EBV positivity. A strong association with EBV was described in 27/87 (31%) of HL; 12/223 (5%) LDCBG and 18/71 (25%) LNK. In HL and LNK, there was also a statistically significant association between EBER-ISH positivity and relapse (p < 0.01). In contrast, there was no significant association between EBV status and age or sex for HL, LCB, or LCT. In the present investigation, no statistical significance was found in the association between EBER-ISH positivity and the overall histological classification of benign and malignant, showing the result of Fisher's exact test of p = 0.207; however, EBER-ISH positivity was concentrated in malignant cases, where 47% s were found to have malignancy results associated with EBV; showing LH (41.7%) in first place and LNK (25.0%) in second place (Table 2 and Table 3).

In the double-blind study by Donzel (2022), conducted at a university hospital in France, a total of 756 cases of lymphoma were recorded in 2020, 89% of which were new diagnoses and 11% were relapses. Of these cases, 575 (76%) were LCB; 110 cases (15%) were HL, and 71 cases (9%) were CLL. Similar data were found in the present study, in which, of 48 cases of malignant pathology, 20/48 were diagnosed with HL, representing the highest number of malignant pathology cases with 41.7% of HL cases associated with EBV (Table 3).

In Wang's study (2021), the EBER--ISH molecular test was used to determine the tumor status in relation to EBV in 134 patients. Forty-six point three percent (62/134) were EBV-positive for EBER-ISH. The EBV-associated HL group had a higher proportion of men than the non-EBV-associated HL group, with 71.0% (44/62) versus 54.2% (39/72), with a p = 0.046 (Wang, 2021). Similar findings were observed in the present study, in which EBV-associated HLs were found to be positive for EBER-ISH in 41.7% of cases (Table 3).

In Karaarslan's study (2015) of LNK cases analyzed between 1997 and 2004 in Turkey, 22.6% of the 62 cases studied were positive for EBER-ISH. Regarding the histological subtype, 100% of extranodal nasal-type LNK were positive for EBER-ISH. Karaarslan's study used Pearson's β tests and Fisher's exact test to compare the associations between histological subtypes and EBER-ISH test positivity, finding statistical significance for the associations. Of the cases with lymph node involvement (n = 53), most were diagnosed by analyzing lymph node biopsies (74.0%) and some cases by analyzing skin biopsies (38.4%). Positive EBER-ISH staining increased to a statistically significant level in cases of extranodal LNK and showed a more robust staining pattern in this subtype of lymphoma (p = 0.019). In contrast to the current study, the result of Fisher's exact test of the association between overall histological classification and EBER-ISH positivity was not statistically significant (p = 0.207).

Tellez concluded in his study that the LLMDA technique provides a sensitive and alternative method for identifying known viral pathogens associated with tumors and could be useful for the future clinical identification of new viral pathogens associated with cancer, as well as EBER-ISH should be used to confirm positive EBV diagnoses in cases where this microbial detection matrix is used (Tellez, 2014). EBV, like many cancer-causing agents, has built-in mechanisms to evade the immune system that hinders the physiological response to antitumorigenesis (Patel, 2022).

Karaarslan concluded in his research that *in situ* hybridization is a sensitive method for identifying EBV in lymphomas and that EBV infection plays an important role in the pathogenesis of T-cell and NK-cell lymphomas in Turkey, findings that are also consistent with those in Western countries.

The findings of the present study highlight the need to strengthen diagnostic capacity in pathology services, particularly through the routine implementation of the EBER-ISH molecular test, whose positivity in a considerable number of cases reinforces its value in identifying EBV in tissues with clinical suspicion of lymphoma. Likewise, training staff in the interpretation and application of this technique is essential to ensure the quality and uniformity of diagnoses.

Among the main limitations of this study are its descriptive and cross-sectional design, which does not allow causal relationships to be established between the presence of EBV and different types of lymphoma. Likewise, non-probabilistic inclusion and dependence on institutional records may have limited the representativeness of the sample and the availability of complementary clinical variables. Nevertheless, the findings provide relevant evidence on the frequency of EBER-ISH biomarker positivity in tissues with clinical suspicion of lymphoma. Future research should increase the sample size, include additional clinical and immunophenotypic variables, and explore the possible correlation between viral load and the aggressiveness of different lymphoma subtypes in order to strengthen our understanding of the role of EBV in the pathogenesis of these neoplasms and guide more accurate diagnostic and therapeutic strategies.

CONCLUSIONS

This study determined a 48% prevalence of positivity for the EBER-ISH biomarker, which shows a considerable presence of Epstein-Barr virus in tissues with clinical suspicion of lymphoma. This finding is relevant because it confirms the frequent involvement of EBV in lymphoid neoplasms, especially Hodgkin's lymphoma and NK-cell lymphoma, reinforcing its importance as an etiopathogenic cofactor. Although no statistically significant association was observed between positivity and overall histological classification, the distribution found provides valuable information for the recognition of local patterns of infection.

These results highlight the need to strengthen routine molecular characterization in the diagnostic evaluation of lymphomas; they also contribute to expanding the evidence on EBV behavior in the Guatemalan population. Therefore, it is recommended that complementary analyses be incorporated to further investigate viral load and its relationship with specific subtypes. As a future line of research, it is suggested that larger multicenter studies with a gre r sample size be conducted to explore robust associations between EBV infection and tumor aggressiveness.

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