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Detection of Herpes Simplex Virus in Lymphoid Tissues Using Immunohistochemistry: Evidence from Western Saudi Arabia

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Abstract: ***Background: Lymphomas, a heterogeneous group of hematologic malignancies, have been linked to several oncogenic viruses. While Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) are well-documented in lymphomagenesis, the potential role of Herpes Simplex Virus (HSV) remains poorly understood. This study investigates the presence of HSV in lymphoma tissues using immunohistochemistry (IHC) in patients from Madinah, Saudi Arabia. Methods: This retrospective cross-sectional study analyzed 66 formalin-fixed paraffin-embedded (FFPE) tissue blocks from lymphoma patients diagnosed between March 2024 and June 2025. Immunohistochemical staining was performed using anti-HSV antibodies to detect viral antigens. Data on patient demographics, sample site, lymphoma subtype, and HSV status were statistically analyzed using SPSS version 21. Results: Of the 66 lymphoma cases, 12 (18.2%) were HSV-positive. HSV was detected more frequently in males (75%) than females (25%). The majority of HSV-positive patients (66.7%) were between 40 and 60 years of age. Hodgkin lymphoma accounted for 4 of the HSV-positive cases, while 8 were found in non-Hodgkin lymphoma. Lymph nodes were the most common site of HSV detection (50%). No HSV positivity was observed in abdominal mass samples. Conclusion: The detection of HSV antigens in a subset of lymphoma cases suggests a possible association between HSV infection and lymphomagenesis. Although a definitive causal relationship cannot be established, these findings highlight the need for further molecular studies to clarify HSV's role in the pathogenesis of lymphoid malignancies, particularly in regions with diverse viral epidemiology such as Western Saudi Arabia.***

Keywords: Herpes Simplex Virus, Lymphoma, Immunohistochemistry, Viral Oncology, Saudi Arabia

1. Introduction

A diverse class of hematologic cancers, lymphomas can arise from B, T, or natural killer (NK) cells. They are often divided into two categories: non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL), each of which has unique clinical, immunophenotypic, and morphological characteristics. Although the exact cause of the majority of lymphomas is still unknown, a number of infectious agents, including viruses like Epstein-Barr virus (EBV), human T-cell lymphotropic virus type 1 (HTLV-1), and Kaposi's sarcoma-associated herpesvirus (KSHV), have been linked to lymphomagenesis (1,2). By encouraging persistent antigenic stimulation, preventing apoptosis, or causing genomic instability, these viruses are believed to have a role in malignant transformation (3).

There are two main kinds of the enveloped double-stranded DNA virus known as Herpes Simplex Virus (HSV). Its main known effects include latency in sensory ganglia. and mucocutaneous infections. Despite the fact that HSV is not as well-known as EBV or KSHV, there is growing interest in its possible function in regulating immunological responses and cellular pathways that, in some circumstances, may aid in the development of cancer (4). Research has shown that HSV DNA is present in a number of cancers, including as lymphomas, oral squamous cell carcinoma, and cervical cancer, indicating a potential link that merits more investigation (5,6).

Immunohistochemistry (IHC), which provides cellular context and spatial resolution, is still a potent and popular technique for identifying viral proteins in tissue samples. IHC has been widely used in the setting of lymphoma to identify viruses like EBV by looking for expression of Epstein-Barr nuclear antigen (EBNA) or latent membrane protein 1 (LMP1). However, there is little and mostly conflicting information about HSV detection in lymphoma tissues using IHC (7). It is conceivable that HSV may exist in lymphoid tissues and might have an impact on the onset or course of lymphoma given its capacity to infect immune cells and survive in latent forms (8).

The population of Western Saudi Arabia, which includes Madinah, is distinct due to its high rate of movement and ethnic diversity, which might have an effect on viral epidemiology. Although HSV is known to be common in the general community, nothing is known about how it relates to lymphomas in this area. Thus, the purpose of this study is to use immunohistochemistry methods to evaluate the presence of HSV in lymphoma tissues. These findings should help us better understand virus-associated lymphoid malignancies in this region and shed light on the potential role of HSV in lymphomagenesis. Understanding the involvement of HSV in lymphoma may broaden our knowledge of virus-associated oncogenesis and guide future diagnostic and therapeutic research in viral oncology. The purpose of this study is to use immunohistochemistry methods to evaluate the presence of HSV in lymphoma tissues.

2. Materials and Methods

Study Design and Participants From:

All patients in Western Saudi Arabia who received a lymphoma diagnosis between March 2024 and June 2025 were included in this retrospective descriptive analysis. The main goal was to use immunohistochemistry methods to determine if lymphoma tissue samples contained Herpes Simplex Virus (HSV).

Study Area and sample size

Histopathology labs located across Madinah, Western Saudi Arabia, provided all tissue samples and related clinical information. This study contained 66 formalin-fixed, paraffin-embedded (FFPE) tissue blocks from individuals who had lymphoma that was verified by histology.

Immunohistochemical Staining Procedure

From pathology archives, FFPE blocks with verified lymphoma diagnoses were obtained. Retrieved and placed on slides coated with poly-L-lysine. Each example employed a single slide for HSV detection. After two hours of incubation at 60°C, the sections were deparaffinized in xylene and rehydrated using a series of graded ethanols (100%, 90%, 70%, and 50%). After being cleaned with distilled water, the slides were placed in a buffer solution with a pH of 7.

Following the Zytomed Systems protocol, the horseradish peroxidase (HRP) one-step polymer anti-rabbit technique was used for immunohistochemical staining. Peroxidase prevent (Cat. No: ZUCO19-100) was used to prevent endogenous peroxidase activity for ten minutes at room temperature. Buffer (Cat. No: ZUC020-500) was then used to wash the area. Citrate buffer (pH 6.0, Cat. No: ZUC028-100) was used to perform antigen retrieval in a water bath at 95°C for 40 minutes. This was followed by chilling at room temperature and further washing.

After applying primary anti-HSV antibodies, they were allowed to sit at room temperature for half an hour. Following incubation, slides were subjected to three 5-minute washes before being treated for 30 minutes with HRP one-step polymer anti-rabbit solution (Cat. No: ZUC053-100). For visualization purposes, DAB high contrast working solution (Cat. No.: DAB 500 plus) was used for five minutes. Sections were blued in tap water, dehydrated in 100% ethanol, cleaned, counterstained for 30 seconds with Mayer's hematoxylin, and then mounted using DPX.

Result Interpretation: A positive reaction was defined by the presence of brown chromogenic deposits at the site of HSV antigen localization.

Data Analysis

Data were entered and analyzed using SPSS version 21. Descriptive statistics, including frequencies and cross-tabulations, were used to assess the distribution of HSV positivity across lymphoma cases.

Ethical Considerations

Approval for data and tissue sample usage was obtained from the participating histopathology laboratories. All procedures were carried out in accordance with local institutional ethical standards and guidelines for research involving human tissue.

Ethical Approval

The study protocol was reviewed and approved by the Research Ethics Committee of AlRayan Colleges, Madinah, Saudi Arabia.

3. Result

This study contained 66 formalin-fixed, paraffin-embedded (FFPE) tissue blocks from individuals who had been diagnosed with lymphoma. Of them, 22 (33.3%) were female and 44 (66.7%) were male. Twelve cases (18.2%) had Herpes Simplex Virus (HSV), including three females (25%), and nine men (75%). The remaining 54 patients, which included 19 females and 35 men, (81.8%) had negative HSV test results.

In terms of age distribution, 18 patients (27.3%) were under 40 years of age, 30 patients (45.5%) were between 40 and 60 years, and 18 patients (27.3%) were older than 60 years. Of the HSV-positive cases, 8 (66.7%) were within the 40–60 age group, 3 (25%) were under 40, and 1 (8.3%) was over 60.

Regarding lymphoma classification, 10 patients (15.2%) had Hodgkin lymphoma (4 males and 6 females), while 56 (84.8%) had non-Hodgkin lymphoma (40 males and 16 females). HSV was identified in 4 Hodgkin lymphoma cases and 8 non-Hodgkin lymphoma cases. Table 1 presents the distribution of HSV status, age groups, and lymphoma subtypes among the study participants Table 1.

Table 1: Distribution of HSV Status, Age Groups, and Lymphoma Types Among the Study Subjects (n = 66)

Variables	Males	Females	Total
HSV Status			
Positive	9	3	12
Negative	35	19	54
Total	44	22	66
Age Group			
< 40	11	7	18
40–60	23	7	30
> 60	10	8	18
Total	44	22	66
Lymphoma Type			
Hodgkin	4	6	10
Non-Hodgkin	40	16	56
Total	44	22	66

When examining the anatomical sites of the tissue samples, HSV was most commonly detected in lymph node specimens, accounting for 6 out of 12 positive cases (50%). Additional HSV-positive findings were noted in 2 nasopharyngeal biopsies (16.7%), and one case each from breast tissue, skin biopsy, tonsillar biopsy, and intestinal

mass (8.3% each). No HSV positivity was found in abdominal masses Table 2.

Table 2: Distribution of HSV (Positive or Negative) by Sex, Age Group, Sample Site, and Lymphoma Type (n = 66)

Variable	HSV Positive	HSV Negative	Total
Sex			
Males	9	35	44
Females	3	19	22
Total	12	54	66
Age Group			
< 40	3	15	18
40–60	8	22	30
> 60	1	17	18
Total	12	54	66
Sample Site			
Lymph node	6	24	30
Abdominal mass	0	20	20
Breast	1	3	4
Skin biopsy	1	1	2
Nasopharyngeal	2	2	4
Tonsil	1	1	2
Intestinal mass	1	3	4
Total	12	54	66
Lymphoma Type			
Hodgkin	4	6	10
Non-Hodgkin	8	48	56
Total	12	54	66

4. Discussion

The current study used immunohistochemistry (IHC) to assess the presence of Herpes Simplex Virus (HSV) in lymphoma tissue specimens from 66 patients in Madinah, Saudi Arabia. A minority of patients tested positive for HSV antigens, according to the data, which may indicate a link between HSV infection and certain lymphoma subtypes. Herpesviruses such as Epstein-Barr Virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) have been extensively researched in connection with lymphomagenesis; however, little is known about HSV's possible involvement in lymphoid cancers. Through processes including oncogene activation and persistent antigenic stimulation, herpesviruses have been found to be cofactors in lymphoid proliferation in previous findings [9, 10].

Particularly in younger individuals and specific histological subtypes, the presence of HSV in lymphoma tissues may indicate either a direct carcinogenic function or the reactivation of a dormant virus as a result of immunosuppression. Numerous investigations have demonstrated that herpesviruses may remain dormant in host tissues and reawaken in situations where the immune system is compromised, such as during chemotherapy or the growth of cancer [11]. Furthermore, concerns regarding viral latency vs lytic infection and its connection to disease behavior and prognosis are brought up by the discovery of HSV antigens in lymphoma tissue. Although causation is still up for debate, these results are in line with recent publications that detected herpesvirus DNA or proteins in neoplastic tissues [12].

Importantly, host variables including immunological state, genetic vulnerability, or environmental exposures may have an impact on the heterogeneity in HSV positive among age groups and lymphoma types in our investigation. Studies from Asia and Africa have reported comparable regional diversity in herpesvirus-associated lymphomas, which is indicative of variations in viral frequency and co-infection patterns [13]. Specifically, by modifying the tumor microenvironment and immune response in concert, co-infection with other herpesviruses, such as EBV or cytomegalovirus (CMV), may exacerbate the pathogenesis of lymphomas [14]. Deeper understanding of these relationships could be possible in future research that uses molecular profiling and multiplex viral detection.

Although this study provides useful information from a previously underrepresented area, it must be noted that it has a number of drawbacks. The findings' generalizability is constrained by the retrospective approach and somewhat small sample size. Furthermore, although the IHC method can identify the expression of viral proteins, it cannot quantify the viral load or distinguish between latent and lytic infections. To verify the existence and function of HSV in lymphomagenesis, more investigation utilizing molecular techniques including polymerase chain reaction (PCR) and next-generation sequencing is necessary. Furthermore, longitudinal research is required to determine if HSV positivity affects the prognosis of lymphoma patients or responsiveness to therapy in any way [15].

5. Conclusion

While the detection of HSV antigens in lymphoma tissues may suggest a possible association, the limitations of the IHC technique and the small sample size necessitate cautious interpretation. Future studies incorporating advanced molecular methods are required to confirm these preliminary findings and assess the prognostic relevance of

HSV in lymphoid malignancies. Multicenter, longitudinal investigations are recommended to further explore its clinical implications.

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