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Latent Membrane Protein 1, CD3, and CD20 Expression Patterns in Lymphomas at a Tertiary Hospital in Southeast, Nigeria

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Abstract

Background: Lymphomas are a heterogeneous group of hematologic malignancies with varying immunophenotypes, clinical presentations, and associations with viral infections such as Epstein-Barr virus (EBV). Immunohistochemistry (IHC) remains a critical tool in the subclassification of lymphomas and in understanding their pathogenesis. The Epstein-Barr virus (EBV), a DNA virus in the herpesvirus family, has been implicated in the etiopathogenesis of various malignancies, particularly in immunocompromised individuals. **Objective:** The aim of this present study is to immunohistochemically characterize lymphoma subtypes using CD3, CD20 and LMP1 markers, assess the distribution of B-Cell and T-Cell lymphomas, and explore the potential association of EBV, via LMP1 expression, with lymphoma cases. **Materials and Methods**: Formalin-fixed, paraffin-embedded tissue sections from confirmed lymphoma cases were stained with CD3, CD20 and EBV-LMP1 antibodies. The immunohistochemical profiles were evaluated, and data were analyzed in relation to age, sex, histological subtype, and anatomical distribution. **Results**: The participants in this study had an average age of 40.75 ± 21.70 years. The mean age at diagnosis was 43.1 ± 21.4 years for Non-Hodgkin's lymphoma and 19.9 ± 9.7 years for Hodgkin's lymphoma. Among the 69 lymphoma cases, 62 (89.9%) were Non-Hodgkin's lymphoma, while 7 (10.1%) were Hodgkin's lymphoma. The highest number of lymphoma cases occurred in the 5th decade of life, with 23 cases (33.3%), while the lowest number was observed in the 1st decade, with only 5 cases (7.2%). A statistically significant association between age and lymphoma was found in this study ($p = 0.013$). In the years

under review, the incidence of lymphoma was higher in male subjects (45 cases, 65.2%) compared to female subjects (24 cases, 34.8%), resulting in a male-to-female ratio of 1.9:1. However, no statistically significant association was found between gender and lymphoma in this study ($p = 0.190$). The results of this study also revealed that 23 cases (33.3%) of lymphoma observed during the review period were of T-Cell lineage, while 46 cases (60.9%) were of B-Cell lineage. A total of 25 cases (36.2%) of lymphoma occurred in both the axilla and cervical regions, while 19 cases (27.5%) were located in the inguinal region. However, no statistically significant association was found between the anatomical site and lymphoma distribution according to cell lineage ($p = 0.748$). Only 1.4% (1/69) of Lymphoma cases were positive for EBV-LMP1, suggesting a limited role for EBV via LMP1 in this population. **Conclusion:** This study confirmed the predominance of Non-Hodgkin's Lymphoma (NHL) across all age groups, particularly among older adults. The study also supports the well-known bimodal age distribution of Hodgkin's Lymphoma (HL), with peaks in young adults and middle-aged individuals. Limited LMP1 expression suggests alternative pathogenetic pathways in EBV-associated lymphomas. These findings underscore the importance of continued investigation into the immunophenotypic and molecular features of lymphomas to enhance diagnostic precision and therapeutic decision-making.

Keywords

Epstein-Barr Virus, Latent Membrane Protein 1, Lymphoma, Hodgkin's Lymphoma, Non-Hodgkin's Lymphoma, Immunohistochemistry

1. Introduction

Lymphomas are a heterogeneous group of neoplasms arising from lymphoid tissues, traditionally classified as Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL), each with distinct biological behaviors, prognoses and responses to treatment. Globally, approximately 287,000 new cases of NHL are diagnosed each year [1], with incidence rates increasing with age and showing a male predominance. Ugwu *et al.*, (2023) recorded a Prevalence of lymphoma of 50.0% patients who presented with lymphadenopathy in Abakiliki, Southeast, Nigeria [2]. Although NHL is relatively uncommon across most African populations, its relative frequency is higher in North and sub-Saharan Africa due to the high incidence of Burkitt's lymphoma (BL), especially in children [3]. Historical data indicate that Ibadan, Nigeria, once recorded one of the highest global incidences of lymphomas [4]. Although BL remains the most common childhood malignancy in this region, its prevalence has decreased over the decades, from 50% in the 1960s to 19.4% in the 1990s—a decline attributed to improved living conditions, better malaria control, and reduced Epstein-Barr virus (EBV) infection rates [5] [6].

EBV, a ubiquitous DNA virus from the herpesvirus family, is implicated in the

pathogenesis of several B-Cell lymphomas, particularly in immunocompromised individuals. These include endemic BL, Hodgkin's lymphoma, and other EBV-associated lymphoproliferative disorders such as HIV-related lymphomas and post-transplant lymphomas [7]. The virus encodes oncogenic proteins such as LMP1 and EBNA2, which activate signaling pathways like NFκB and JAK/STAT, and dysregulate cell survival and proliferation, contributing to malignant transformation [8].

More than 90% of the global population carries latent EBV infection [9], with earlier exposure often observed in developing regions due to environmental and cultural factors [10] [11]. Ahmad *et al.*, (2022), in a recent research, documented that the Prevalence of Epstein Barr virus infection amongst lymphoma patients in Nigeria is 13.4% [12]. Epidemiological and genetic studies have established a strong association between EBV and several lymphoma subtypes, including BL, diffuse large B-Cell lymphoma (DLBCL), plasmablastic lymphoma, and HL [13]-[15].

Recent observations indicate an increasing prevalence of lymphomas in our local environment. However, a thorough review of existing literature reveals a scarcity of published data on the prevalence of lymphomas and an absence of studies detailing the clinicopathologic patterns and treatment outcomes in our center. Regional data on the burden of lymphoma are crucial, as variations in incidence and disease characteristics may exist even within the same country. Understanding these regional trends is essential not only for informing healthcare providers and guiding timely and accurate diagnosis and management but also for assisting governmental and public health agencies in effective healthcare planning and resource allocation. Despite extensive research, the precise mechanisms by which EBV contributes to lymphomagenesis remain incompletely understood, particularly due to variability in viral gene expression and population-specific patterns. This study aims to bridge this knowledge gap by investigating EBV's role in lymphoma pathogenesis through immunohistochemical analysis of LMP1 expression across lymphoma subtypes. By doing so, it seeks to elucidate EBV's oncogenic influence and offer insights into potential diagnostic and therapeutic strategies, especially in high-prevalence settings.

2. Materials And Methods

2.1. Study Design

This was a 10-year retrospective study that comparatively analyzed lymphoma in previously diagnosed samples of formalin fixed, paraffin wax embedded Lymphoma tissue blocks from 2013 to 2023 retrieved from the Histopathology Department of Nnamdi Azikiwe University Teaching Hospital Nnewi. Also retrieved from the available records were patients' demographic data. Ethical approval for the study was obtained from the Ethics Committee (NAUTH/CS/66/VOL.16/VER.3/02/2023/02) of the hospital before commencement of the study.

2.2. Selection of Sample Blocks

Paraffin wax embedded tissue blocks of subjects who were previously diagnosed and reported with various histological subtypes and grades of lymphoma in Histopathology Department, Nnamdi Azikiwe University Teaching, Nnewi, Anambra state starting from 2013 to 2023 were used in this study. The paraffin wax embedded tissue blocks that were selected comprised all age groups and both sexes.

2.3. Inclusion Criteria

All formalin fixed paraffin wax embedded blocks of lymph node tissues of subjects who had been previously confirmed lymphoma positive by the pathologist from 2013 to 2023 with detailed records were included in this study.

2.4. Exclusion Criteria

The following groups of subjects were excluded from this study:

1) Formalin fixed paraffin wax embedded blocks of lymph node tissues of subjects previously confirmed lymphoma positive by the pathologist from 2013 to 2023 but without detailed records.

2) Formalin fixed paraffin wax embedded blocks of lymph node tissues of subjects previously confirmed lymphoma positive by the pathologist before 2013.

3) Samples that were tiny and would not be enough for analysis.

4) Samples that have been eaten by rodents and damaged tissue blocks.

2.5. Laboratory Analysis

The archived paraffin embedded tissue blocks of the subjects were retrieved. The tissue blocks were trimmed and serial sections of 4 μ thickness cut on a Rotary microtome (HM340E Thermo Scientific. Massachusetts, United States of America). The tissue ribbons produced were floated on tissue flotation bath set at 50 ˚C. The floated sections were picked up on clean glass slides each, allowed to air dry, and then stained with haematoxylin and eosin staining technique. The stained slides of the subjects were coded for blind assessment and were reviewed by pathologists to reconfirm the diagnosis.

The sections were taken to water and stained in Erlich's haematoxylin for 20 minutes. The stained sections were rinsed in tap water, differentiated in 1% acid alcohol for 15 seconds and then rinsed in tap water. The sections were blued in Scott's tap water and stained in 1% aqueous eosin solution for 1minutes. The sections were washed in running tap water for 30 seconds. The stained sections were dehydrated in ascending grades of alcohol, cleared and mounted using DPX (Di-N-Butyl Phthalate in Xylene) and glass cover slip [16]. Care was taken to avoid air bubbles. Photomicrographs were taken using Olympus microscope (BHTU. New York Microscope Company).

The immunohistochemical method used in this present study is the Avidin Biotin Complex (ABC) method also referred to as Avidin Biotin Immunoperoxidase

method. The principle is based on a simple indirect antigen and antibody reaction. The biomarkers (antigens in the tissues), combined with their corresponding antibodies (CD3, CD20 and LMP1) to form a complex which is coupled with a secondary antibody and in the presence of an immunogen (3,3diaminobenzidine) forms a brown colour complex. The intensity of this brown colour complex is directly proportional to the concentration of the biomarkers in the tissues [17].

One block of formalin-fixed, paraffin-embedded tumor tissue was selected per case and 4 micrometer thick section was cut, deparaffinized and rehydrated by passing through 2 changes of xylene, then 4 changes of descending grades of alcohol (100%, 90%, 80%, 70%) and finally to water. Antigen retrieval was performed by immersing and heating sections on a citrate buffer solution of pH 6.0 using the microwave at power 100 for 15 minutes. The sections were equilibrated gradually with cool water to displace the hot citrate buffer for at least 5 min for the section to cool. Endogenous peroxidase blocking was done on the sections by simply covering section with 3% hydrogen peroxide (H_2O_2) for 15 min. Sections were washed 2 times in phosphate buffered saline (PBS) for 3 minutes each. The slides were incubated with protein blocker (avidin) for 20 mins to prevent non-specific binding and then washed in phosphate buffered saline (PBS). Endogenous biotin in tissue was blocked using biotin for 15 mins and washed with PBS [17].

Sections were then incubated with primary antibodies; mouse monoclonal antibodies against CD3, CD20 and LMP1 proteins in a humid chamber for 1 hour at room temperature. Excess antibodies will be washed off with PBS and a secondary antibody (LINK) was applied on sections for 15 mins. Sections were washed and the (LABEL) horseradish peroxidase (HRP) was applied to the sections for 15 mins. A working DAB (3.3'-diaminobenzidine) solution was made up by mixing 1 drop (20 microns) of the DAB chromogen to 1 ml of the DAB substrate. This working solution was applied on sections after washing off the HRP with PBS for at least 5 mins. The brown reaction begins to appear at this moment especially for a positive target. Excess DAB solution and precipitate were washed off with water [17].

Sections were counterstained with haematoxylin solution for 5 min and blued briefly. Sections were dehydrated in alcohol, cleared in xylene and mounted in DPX. Stained sections were reviewed for nuclear reactivity. Micrographs were taken using Olympus microscope [17].

2.6. Statistical Analysis

The data obtained were analyzed using SPSS (Statistical Package for Social Sciences) 20.0. Fisher Exact test and chi-square test were used to determine statistical differences and the association between variables. A p-value ≤ 0.05 was considered statistically significant in all statistical analyses.

3. Results

Over the period from 2013 to 2023, a total of 91 lymphoma cases were recorded. However, 7 of these cases were excluded due to the unavailability of tissue blocks,

while 15 cases were excluded because of insufficient tissue in the paraffin blocks, which was attributed to tissue degradation and suggestive of potential issues with sample preservation. Consequently, 69 cases of lymphoma tissue blocks were selected for immunohistochemical staining.

Table 1 and Figure 1 present the distribution of lymphoma cases among patients at NAUTH, categorized into Hodgkin's and Non-Hodgkin's lymphoma by age group. Among the 69 subjects studied immunohistochemically, the youngest was 4 years old, while the oldest was 82 years old. The mean age at diagnosis was 43.1 ± 21.4 years for Non-Hodgkin's lymphoma and 19.9 ± 9.7 years for Hodgkin's lymphoma. According to Table 1, the highest number of lymphoma cases (23 cases, or 33.3%) occurred in the >50 years age group, with all cases being Non-Hodgkin's lymphoma. Five cases (7.2%) were observed in the 1 - 10 years age group, while 6 cases (8.7%) were in the 21 - 30 years age group. The 31 - 40 years age group accounted for 8 cases (11.6%), and the 11 - 20 years and 41 - 50 years age groups had 13 (18.8%) and 14 cases (20.3%), respectively.

Table 1. Age group distribution of lymphomas cases into Hodgkin's and Non-Hodgkin's lymphoma.

Age group (Years)	Non-Hodgkin's Lymphoma n (%)	Hodgkin's Lymphoma n (%)	TOTAL n (%)
1 - 10	4 (80)	1 (20)	5 (7.2)
11 - 20	11 (84.6)	2 (15.4)	13 (18.8)
21 - 30	4 (66.7)	2 (33.3)	6 (8.7)
31 - 40	6 (75.0)	2 (25.0)	8 (11.6)
41 - 50	14 (100.0)	0 (0.0)	14 (20.3)
>50	23 (100.0)	0 (0.0)	23 (33.3)
TOTAL	62 (89.9)	7 (10.1)	69 (100.0)

X^2 (5, N = 69) = 10.786*, p = 0.013; Keys: n = absolute number; % = percentage, * = Fisher's Exact Text.

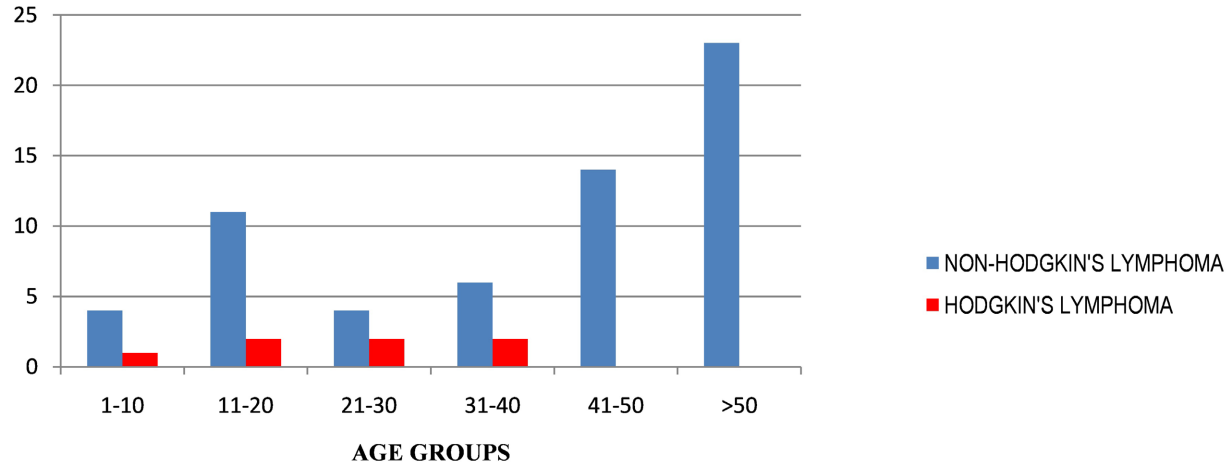

Figure 1. Age distribution of lymphomas cases into Hodgkin's and Non-Hodgkin's lymphoma.

Table 2 shows the gender distribution of lymphomas cases into Hodgkin's and

non-Hodgkin's lymphoma. Out of the 69 cases of lymphoma, 62 (89.9%) were Non-Hodgkin's Lymphomas whereas 7 (10.1%) were Hodgkin's Lymphoma. 42 (67.7%) cases of Non-Hodgkin's Lymphomas were of male subjects while 20 (32.3%) were of female subjects. The incidence of Lymphoma within the year under review was higher in male subjects than female subjects with male to female ratio of 1.9:1.

Table 2. Gender distribution of lymphomas cases into Hodgkin's and Non-Hodgkin's lymphoma.

Gender	Non-Hodgkin's Lymphoma n (%)	Hodgkin's Lymphoma n (%)	Total n (%)
Male	42 (93.3)	3 (6.7)	45 (65.2)
Female	20 (83.3)	4 (16.7)	24 (34.8)
Total	62 (89.9)	7 (10.1)	69 (100.0)

X^2 (1, N = 69) = 1.717, p = 0.190; Keys: n = absolute number; % = percentage.

Table 3 shows the distribution of lymphoma cases among patients in NAUTH into B-Cell and T-Cell lymphoma with respect to age group. From the result of this present study, it was discovered that 23(33.3%) of the lymphoma cases seen in NAUTH within the years under review were of T-Cell linage as shown in Figure 2. 46(66.7%) cases of the lymphomas were of B-Cell linage. Out of the 23 cases of the T-Cell Lymphomas, age groups 41 - 50 and >50 years had the highest number of cases 8 (34.8%) each, while age groups 1 – 10 years and 31 - 40 years had the lowest number of cases with 1 (4.3%) each. The highest number of B-Cell Lymphoma cases 15 (32.6%) was seen among the age group of >50 years. Lymphoma cases of B-Cell lineage were uncommon, with only 4 cases (8.7%) each in the 1 - 10 and 31 - 40 age groups.

Table 3. Distribution of lymphomas cases into B-Cell and T-Cell according to age group.

Age Group (Years)	T-Cell Lymphoma n (%)	B-Cell Lymphoma n (%)	Total n (%)
1 – 10	1 (20.0)	4 (80.0)	5 (7.2)
11 – 20	3 (23.1)	10 (76.9)	13 (18.8)
21 – 30	2 (33.3)	4 (66.7)	6 (8.7)
31 – 40	1 (12.5)	7 (87.5)	8 (11.6)
41 – 50	8 (57.1)	6 (42.9)	14 (20.3)
>50	8 (34.8)	15 (65.2)	23 (33.3)
Total	23 (33.3)	46 (66.7)	69 (100.0)

X^2 = (5, N = 69) = 5.681*, p = 0.338; Keys: n = absolute number; % = percentage, * = Fisher's Exact Text.

Table 4 shows the distribution of lymphoma cases into B-Cell and T-Cell with respect to Gender. Within the years under review, 45 (65.2%) of all lymphoma cases were of male gender, and 24 (34.8%) were of female gender according to

Table 4. Out of the 46 cases of B-Cell lymphomas, 28 (60.1%) and 18 (39.1%) were of male and female respectively, whereas out of the 23 cases of T-Cell lymphomas, 17 (73.9%) and 6 (26.1%) were of male and female respectively.

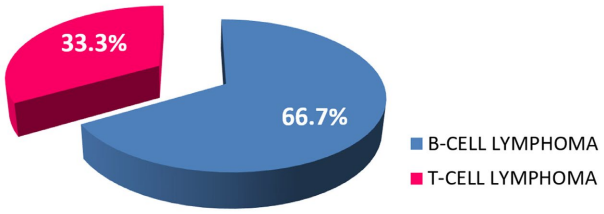

Figure 2. Distribution of Lymphoma cases into B-Cell and T-Cell.

Table 4. Distribution of lymphomas cases into B-Cell and T-Cell according to gender.

Gender	T-Cell Lymphoma n (%)	B-Cell Lymphoma n (%)	Total n (%)
Male	17 (37.8)	28 (62.2)	45 (65.2)
Female	6 (25.0)	18 (75.0)	24 (34.8)
Total	23 (33.3)	46 (66.7)	69 (100.0)

$X^2 = (1, N = 69) = 1.150$, $p = 0.212$; Keys: n = absolute number; % = percentage.

Table 5 presents the distribution of lymphoma cases based on tissue type and anatomical site. In the present study, the axillary region accounted for 25 cases (36.2%) of lymphoma, with Non-Hodgkin's Lymphoma (NHL) comprising the majority (23 cases, 92.0%). Similarly, 25 cases (36.2%) were observed in the cervical region, of which 20 cases (80.0%) were NHL. The inguinal region showed 19 cases (27.5%), with NHL again representing the predominant subtype.

Table 5. Distribution of lymphoma cases with respect to the nature of tissue/anatomical site.

Nature of Tissue/Anatomical Site	Non-Hodgkin's Lymphoma n (%)	Hodgkin's Lymphoma n (%)	Total n (%)
Axillary Lymph Node	23 (92.0)	2 (8.0)	25 (36.2)
Cervical Lymph Node	20 (80.0)	5 (20.0)	25 (36.2)
Inguinal Lymph Node	19 (100.0)	0 (0.0)	19 (27.5)
TOTAL	62 (89.9)	7 (10.1)	69 (100.0)

$X^2 = (2, N = 69) = 4.396^*$, p = 0.115; Keys: n = absolute number; % = percentage, * = Fisher's Exact Text.

According to Table 6, both the axillary and cervical lymph nodes exhibited identical distributions of lymphoma subtypes, with B-Cell lymphomas accounting for 17 cases (68.0%) and T-Cell lymphomas for 8 cases (32.0%) at each site. In the inguinal lymph nodes, T-Cell lymphomas were observed in 7 cases (36.8%), while B-Cell lymphomas were more common, with 12 cases (63.2%). Overall, B-Cell lymphomas demonstrated the highest percentage of occurrence across all anatomical sites in this study.

Table 6. Distribution of Lymphomas into T-Cell and B-Cell Lineage According to the Nature of Tissue/Site of Lymph Node.

Nature of Tissue/Anatomical Site	T-Cell Lymphoma n (%)	B-Cell Lymphoma n (%)	Total n (%)
Axillary Lymph Node	8 (32.0)	17 (68.0)	25 (36.2)
Cervical Lymph Node	8 (32.0)	17 (68.0)	25 (36.2)
Inguinal Lymph Node	7 (36.8)	12 (63.2)	19 (27.5)
Total	23 (33.3)	46 (66.7)	69 (100.0)

$X^2 = (2, N = 69) = 0.145$, p = 0.900; Keys: n = absolute number; % = percentage.

Table 7. Distribution of type of lymphomas according to T-Cell and B-Cell lineage.

Type of Lymphoma	T-Cell n (%)	B-Cell n (%)	Total
Non-Hodgkin's Lymphoma	23 (37.1%)	39 (62.9%)	62 (89.9%)
Hodgkin's Lymphoma	0 (0.0%)	7 (100.0%)	7 (10.1%)
Total	23 (33.3%)	46 (66.7%)	69 (100.0%)

$X^2 = (1, N = 69) = 3.895$, p = 0.050; Keys: n = absolute number; % = percentage.

Table 8. Latent membrane protein 1 (LMP1) expression patterns of lymphomas cases.

Type of Lymphoma	LMP1		Total
	Positive	Negative	
Non-Hodgkin's Lymphoma	1 (1.6%)	61(98.4%)	62 (89.9%)
Hodgkin's Lymphoma	0 (0.0%)	7 (100.0%)	7 (10.1%)
Total	1 (1.4%)	68 (98.6%)	69 (100.0%)

$X^2 = (1, N = 69) = 0.115$, p = 0.899; Keys: n = absolute number; % = percentage.

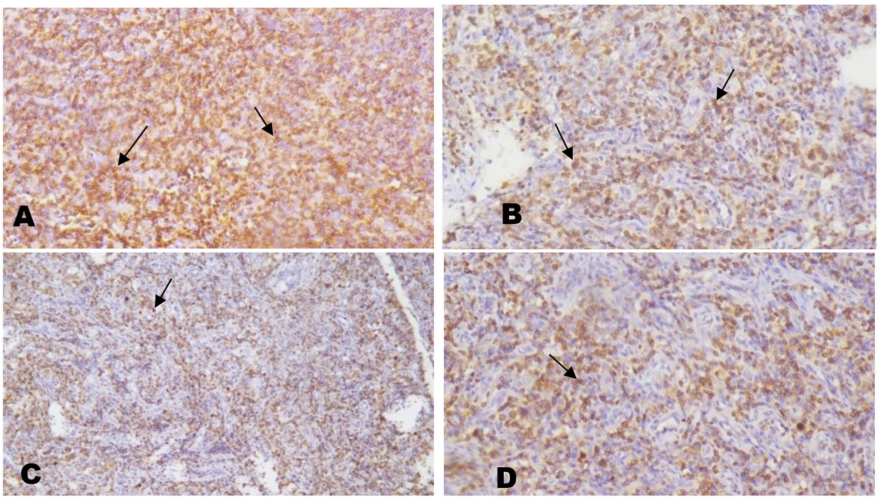

Figure 3. Immunohistochemical staining of lymphoma cases with CD 3 antibody. (A)-(D) shows positive staining of T-Cells at the paracortical area. (×100 magnification).

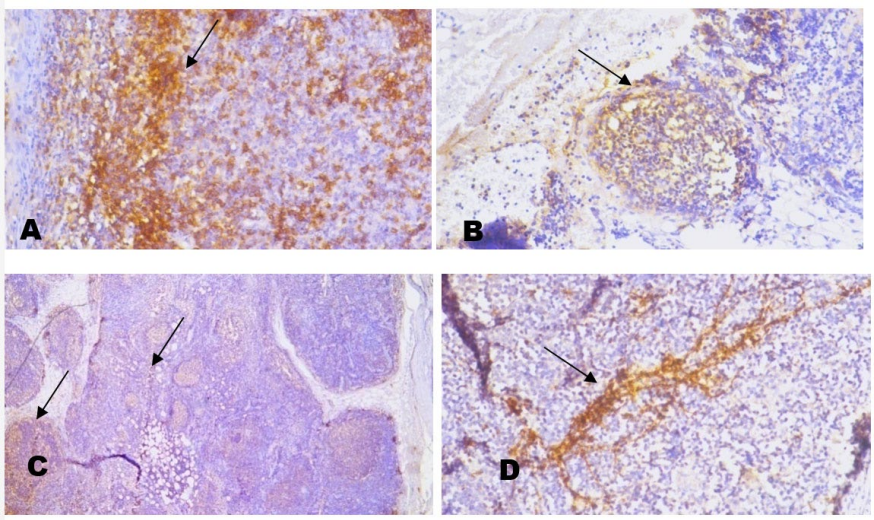

Figure 4. Immunohistochemical staining of lymphoma cases with CD 20 and EBV-LMP1 antibodies. (A)-(C) shows positive staining of B-Cells at the follicular area. (D) shows bositive staining for LMP1. (×100 magnification).

Based on the findings of this study, 23 (37.1%) of the 62 Non-Hodgkin's Lymphoma cases diagnosed at NAUTH during the study period were of T-Cell lineage, while 39 cases (62.9%) were of B-Cell lineage. As shown in **Table 7**, all 7 cases (100.0%) of Hodgkin's Lymphoma were of B-Cell lineage, with no cases of T-Cell lineage observed. As presented in **Table 8**, the study observed that only 1 (1.6%) of the 62 cases of Non-Hodgkin's Lymphoma exhibited positivity for the EBV-LMP1 antibody, while none of the Hodgkin's Lymphoma cases tested positive.

4. Discussion

This study aimed to classify lymphoma cases into B-Cell and T-Cell subtypes using CD20 and CD3 antibodies, determine the expression of latent membrane protein (LMP1), and evaluate potential associations between EBV and lymphoma. The trends observed in this study align with established epidemiological patterns of lymphoma incidence. Non-Hodgkin's Lymphoma NHL is the predominant type across all age groups, with a steady increase that peaks in individuals over 50. This aligns with previous studies indicating that NHL is more common in older adults due to factors such as genetic predisposition, immunodeficiency, and environmental exposures [18] [19]. The rising incidence in older age groups may be attributed to the accumulation of genetic mutations and age-related immune dysfunction. In contrast, Hodgkin's Lymphoma (HL) remains relatively low across all age groups, with only a modest increase in older adults. This supports its well-known bimodal age distribution, with incidence peaks in young adults (early 20 s) and middle-aged individuals (mid-60s) [20], unlike NHL, which is driven by multiple genetic and environmental factors. These findings reinforce that lymphoma frequency increases with age, particularly for NHL, while HL remains less common in comparison.

Several reports have indicated that NHL affects more males than females and the incidence increases with age [21]. The male to female ratio is similar to that observed in previous studies across the world [22]. The gender distribution analysis (**Table 2**) presents the gender distribution of 69 lymphoma cases, of which 62 (89.9%) were diagnosed as Non-Hodgkin's Lymphoma (NHL) and 7 (10.1%) were diagnosed as Hodgkin's Lymphoma (HL). Males comprised 65.2% (n = 45) of the total cases, with 42 (93.3%) having NHL and 3 (6.7%) having HL. Females, on the other hand, made up 34.8% (n = 24) of the cases, with 20 (83.3%) diagnosed with NHL and 4 (16.7%) with HL. Although these figures indicate a somewhat higher proportion of Hodgkin's Lymphoma among females (16.7% vs. 6.7% in males), the chi-square test ($p = 0.190$) shows that this difference is not statistically significant. In summary, Non-Hodgkin's Lymphoma is the predominant subtype for both genders, with no significant gender-based difference in the distribution of Hodgkin's versus Non-Hodgkin's Lymphoma. The male predominance though had been noted in previous studies cannot be easily explained except by exposure to some occupational carcinogens peculiar to occupations predominated mainly by males [22]. Although HL appeared slightly more frequent in females, statistical analysis demonstrated no significant gender-based differences. This data is similar to that reported by [21] suggesting a male predominance in lymphomas, particularly NHL, but without substantial sex-linked variations in HL occurrence.

The characterization of lymphomas into B-Cell and T-Cell lineages (**Table 3** and **Table 4**) is critical for understanding their immunophenotypic distribution. The findings of this present study indicate that B-Cell lymphomas are more prevalent than T-Cell lymphomas, particularly in younger individuals. This is similar to various reports from around the world and Nigeria [23] [24]. Although T-Cell lymphomas were less common in this research, it remains significant in lymphoma pathology, especially in adult and elderly populations. According to John *et al.*, (2018), T-Cell lymphomas represent about 3.1% of all NHL cases in Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria [24]. The 33.3% prevalence of T-Cell lymphoma in this study is higher than the global average. This could be due to regional factors or specific patient characteristics in NAUTH.

The distribution of T-Cell lymphoma showing the highest frequency in the 41-50 years and >50 years age groups (34.9% each) is supported by findings from Zhang *et al.*, (2019), who noted that the incidence of T-Cell lymphoma tends to increase with age [25]. The aging immune system may become less efficient at detecting and eliminating malignant T-cells, which could contribute to the increased prevalence of T-Cell lymphoma in older populations. Younger age groups such as 1 - 10 years and 21 - 30 years exhibiting the lowest frequency of T-Cell lymphoma (4.3% each) aligns with general findings that T-Cell lymphoma is much rarer in children and young adults. A study by Horwitz *et al.*, (2015) also found that pediatric and young adult populations show lower incidences of T-Cell lymphoma, with the disease becoming more common in later stages of life [26]. Older indi-

viduals tend to accumulate mutations in both T-cells and B-Cells over time, which can lead to lymphoma development. The increase in lymphoma cases with age is generally attributed to immune system aging (immunosenescence), which weakens the body's ability to control abnormal cells, especially B-Cells and T-cells. This leads to an accumulation of malignant cells. As noted by Mancuso *et al.*, (2018), this decline in immune function is a primary factor contributing to the higher incidence of lymphomas in elderly populations [27]. These findings highlight the importance of age-specific screening and management strategies in lymphoma care, particularly for older populations.

From the study, 62 cases of NHL were observed, of which 37.1% were of T-Cell lineage and 62.9% were of B-Cell lineage. The dominance of B-Cell lineage in NHL is well documented in literature, with B-Cell lymphomas constituting about 85% - 90% of all NHL cases, and T-Cell lymphomas representing a smaller proportion (typically around 10% - 15%) [28]. This matches the findings in this present study, where B-Cell lymphomas (62.9%) are more prevalent than T-Cell lymphomas (37.1%). In this study, 7 cases of Hodgkin's Lymphoma (HL) were observed, all of which were of B-Cell lineage. The result that all cases were B-Cell lineage aligns with the general understanding of HL's pathology [18]. Hodgkin's Lymphoma is characterized by the presence of Reed-Sternberg cells, which are generally of B-Cell origin [18]. Unlike NHL, where both B-Cell and T-Cell lymphomas are common, HL is almost exclusively a B-Cell disease, particularly in the nodular sclerosis and mixed cellularity subtypes [18]. The incidence of HL is generally lower than that of NHL, but it still remains a significant concern. The dominance of B-Cell origin in HL has been consistent in multiple studies, and the result of this result is not an outlier in this context. A study conducted by Onwubuya *et al.*, (2015) in Nigeria observed a predominance of B-Cell NHL, similar to findings in this study, though the exact proportions of T-Cell vs. B-Cell cases might differ based on the sample population and diagnostic methods used [23]. This could indicate regional patterns in the pathogenesis of NHL.

Importantly, LMP1 expressions were detected in only one NHL case, with no HL cases testing positive for LMP1 expression. This finding aligns with studies indicating that not all EBV-associated lymphomas demonstrate detectable LMP1 expression [29]. The absence of a significant association between LMP1 and lymphoma subtypes in this study suggests that EBV may not play a central role in lymphomagenesis in this population or that alternative viral or genetic mechanisms are involved [30]. The immunohistochemical images (**Figures 3(A)-(D)**) confirm the presence of T-cell lineage in lymphoid infiltrates, as evidenced by strong CD3 expression in the paracortical region. This distribution pattern is characteristic of T-cell–associated lymphoproliferative disorders and aligns with previous research demonstrating the diagnostic utility of CD3 in distinguishing T-Cell from B-Cell lymphomas [18]. **Figures 4(A)-(C)** demonstrate positive CD20 staining of B-Cells localized within the follicular areas. **Figure 4(D)** shows positive immunoreactivity for EBV-LMP1. The observed staining patterns further

validate the immunophenotypic classification applied in this study.

This study successfully achieved its aim of immunohistochemically characterizing lymphomas and evaluating their relationship with EBV through LMP1 expression. The findings confirm the predominance of NHL and B-Cell lineage while showing no significant correlation between lymphoma type and EBV infection via LMP1 expression. The lack of a significant gender or anatomical site preference suggests that lymphoma presentation in this cohort is broadly consistent with global trends [23] [29].

5. Conclusions

This study successfully employed immunohistochemical techniques to classify lymphoma cases into B-Cell and T-Cell subtypes, evaluate the expression of latent membrane protein 1 (LMP1), and investigate the potential association between Epstein-Barr virus (EBV) and lymphoma pathogenesis. The findings corroborate established epidemiological patterns, notably the predominance of Non-Hodgkin's Lymphoma (NHL) across all age groups, particularly among older adults. The bimodal age distribution characteristic of Hodgkin's Lymphoma (HL), with incidence peaks in young adults and middle-aged individuals, was also reaffirmed.

A clear male predominance was observed in NHL cases, while no significant gender-based differences were noted in the distribution of HL versus NHL. B-Cell lymphomas were more prevalent than T-Cell lymphomas, particularly among younger individuals, with no statistically significant age-related increase in T-Cell lymphoma incidence. Anatomically, NHL was more frequently identified across all lymph node sites, though no distinct site-based predilection was observed for either NHL or HL.

LMP1 expression was observed in only one NHL case and was entirely absent in all HL cases, indicating a potentially limited involvement of EBV, specifically through the LMP1 pathway, in lymphoma pathogenesis within this cohort. These findings are consistent with previous studies suggesting that LMP1 is not consistently expressed in EBV-associated lymphomas, pointing to the likelihood of other viral or genetic factors contributing to disease development [23]. There is a need for further studies to substantiate these findings.

Overall, the study highlights distinct immunophenotypic and epidemiological differences between B-Cell and T-Cell lymphomas within both NHL. The predominance of B-Cell lineage in NHL, coupled with the notable presence of T-Cell cases, underscores the need for further investigation into the biological and clinical implications of T-Cell lymphomas. Given the more aggressive clinical course of T-Cell lymphomas, these findings emphasize the importance of precise subtype classification for prognostication and treatment planning. Future studies with larger sample sizes, as well as integrated molecular and genetic profiling, are warranted to deepen understanding of lymphoma pathogenesis and improve diagnostic and therapeutic approaches.

6. Recommendations

1) Future studies should incorporate molecular and genetic profiling techniques, such as PCR and next-generation sequencing, to complement immunohistochemical findings and provide deeper insights into lymphoma pathogenesis and EBV-related oncogenesis.

2) To enhance the statistical power and generalizability of findings, similar studies should be conducted using larger sample sizes across multiple institutions and geographic regions.

3) Although LMP1 expression was limited, routine EBV testing (including EBER in situ hybridization) should be considered, especially in younger patients and aggressive lymphomas, to better understand the virus's role in disease development.

4) The routine use of immunohistochemistry for CD markers (e.g., CD20, CD3, CD5, CD30) should be emphasized in diagnostic protocols to ensure accurate subclassification of lymphomas and guide treatment decisions.

5) Given the relatively aggressive nature and clinical challenges associated with T-Cell lymphomas, targeted studies are recommended to explore their unique biology, treatment response, and prognostic markers.

6) Investigating the long-term outcomes of patients with EBV-negative lymphomas may shed light on alternative oncogenic mechanisms and identify potential therapeutic targets.

Future research should integrate clinical parameters (e.g., staging, treatment outcomes, survival data) to assess how immunophenotypic and EBV-related profiles influence prognosis and therapy response.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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