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Original Article

Immunohistochemical spectrum of lymphoma in Patan Hospital: a two-year retrospective study

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ABSTRACT

Background: Lymphomas are a heterogeneous group of disorders arising from lymphoid tissue and are categorized based on histological and immunophenotypical features. Accurate subclassification of lymphoma is essential for appropriate management and prognostication. This study aimed to analyze the distribution of lymphoma cases at Patan Hospital using immunohistochemistry and to categorize their subtypes according to the 2017 WHO classification system.

Materials and Methods: This retrospective cross-sectional study was conducted in the Department of Pathology, Patan Hospital, from July 2023 to July 2025. All lymphoma cases with complete immunohistochemical evaluation were included. Cases were classified according to the 2017 WHO classification system.

Results: A total of 51 cases were analyzed, with patients ranging from 7 to 92 years of age and a male-to-female ratio of 2:1. Non-Hodgkin lymphoma accounted for 92.2% of cases, predominantly B-cell lymphomas (87.2%). Diffuse Large B Cell Lymphoma was the most common subtype (56.1%). T-cell lymphomas constituted 12.8% of Non Hodgkin Lymphoma. Nearly equal extranodal (51%) and nodal (49%) involvement was observed. The gastrointestinal tract and cervical lymph nodes were the most common extranodal and nodal sites respectively. Hodgkin lymphoma accounted for 7.8% of cases, all of which were of the classic type.

Conclusions: Diffuse large B-cell lymphoma was the most commonly diagnosed lymphoma at Patan Hospital, with B-cell non-Hodgkin lymphomas representing the majority of cases. Immunohistochemistry was essential for accurate subclassification and prognostic assessment, providing valuable baseline data for future regional studies.

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INTRODUCTION

Lymphoma represents a broad group of hematological malignancies with diverse etiological factors, clinical presentations, histological features, molecular profiles, and therapeutic approaches.[1] Lymphomas are broadly categorized into Hodgkin Lymphoma (HL) and Non-Hodgkin Lymphoma (NHL). The World Health Organization (WHO) Classification of Lymphoid Neoplasms is based on morphology, immunophenotype, clinical presentation, and genetic alterations, all of which are essential for accurate diagnosis and appropriate management.[2]

Globally, lymphoma constitutes a significant health burden. According to the Global Cancer Report 2020, there were 627,400 (3.2%) new cases of lymphoma worldwide in 2020, representing an 85.98% increase compared to 2000. In the same year, lymphoma-related deaths numbered 283,200 (2.8%), reflecting a 47.8% rise since 2000.[3] In Nepal, NHL was the 12th most commonly diagnosed cancer in 2020, indicating the growing local relevance of the disease.[4]

Given this increasing burden, accurate subclassification of lymphoma is essential for guiding therapeutic decisions and predicting clinical outcomes. The classification of lymphomas has evolved with the incorporation of various novel immunohistochemical markers that aid in diagnosis and prognostication. At Patan Hospital, the introduction of immunohistochemistry (IHC) in 2023 has significantly enhanced our ability to classify lymphoid neoplasms, differentiate between B-cell and T-cell lymphomas, identify specific subtypes, and detect markers relevant for targeted therapies. This advancement has not only improved diagnostic accuracy but also allowed for more personalized treatment strategies, ultimately improving patient care. Therefore, this study aims to analyze the distribution of lymphoma cases at Patan Hospital using immunohistochemistry and to categorize their subtypes according to the 2017 WHO classification system.

MATERIAL AND METHODS

This retrospective cross-sectional study was conducted in the Department of Pathology, Patan Hospital, on samples received between July 2023 and July 2025. Ethical approval was obtained from the Institutional Review Committee of Patan Hospital. All diagnosed lymphoma cases during the study period were included. Cases with incomplete immunohistochemical evaluation were excluded. Formalin-fixed, paraffin-embedded tissue sections were stained with hematoxylin and eosin stain (H&E) and examined for morphological assessment and initial diagnosis. IHC was performed using standard protocols, and the antibody panels were selected based on morphological findings. The markers included CD45, CD19, CD20, CD3, CD5, CD4, CD8, CD10, BCL6, MUM1, BCL2, MYC, CD23, Cyclin D1, SOX11, TdT , CD30, CD15, PAX5,OCT2/BOB1, EBV-LMP, ALK and ki-67. All cases were classified according to the WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues. Diffuse large B cell lymphoma (DLBCL) cases were further categorized into germinal centre B cell (GCB) and non-GCB types using the Hans algorithm, and double expressor status was also assessed.

Data were entered into Microsoft Excel and analyzed descriptively. Non-numerical variables were expressed as frequencies and percentages, while numerical variables were summarized using the mean.

RESULTS

The study population consisted of 51 patients ranging in age from seven to 92 years. There were eight pediatric cases (age ≤18 years), with a mean age of 14 years. The remaining 43 patients were adults, with a mean age of 61.44 years. The cohort included 34 males and 17 females, yielding a male-to-female ratio of 2:1. The distribution of nodal and extranodal sites is summarized in Table 1. Extranodal involvement predominated (51%), with the most common site being the gastrointestinal tract. Nodal involvement comprised 49%, most commonly affecting the cervical lymph nodes.

NHL accounted for 47 cases (92.16%), while HL was identified in 4 cases (7.84%), as shown in Table 2. Among the NHL, only two cases were precursor lymphoid neoplasms, accounting for 3.92% of the cases, with one case each of B- and T-lymphoblastic leukemia/lymphoma. Both cases occurred in 16-year-old patients. The majority of NHL cases were mature lymphoid neoplasms, comprising 96.08% of cases.

Among the NHL cases, the majority were B-cell lymphomas (n=41, 87.2%) while T-cell lymphomas accounted for 12.8%. Lymphoma subtypes are detailed in Table 2. DLBCL was the most common B-cell subtype (n=23, 56.1%), with 65.2% classified as GCB type and 34.8% as non-GCB type. (fig.1) Double expressor status for MYC and BCL2 was evaluated in 15 cases of DLBCL, of which six were positive. Mantle cell lymphoma (MCL) and marginal zone lymphoma (MZL) were each identified in five cases (12.2%). Among the MCL, two were of the blastoid variant. Most MZL (four of five) were of the extranodal type.

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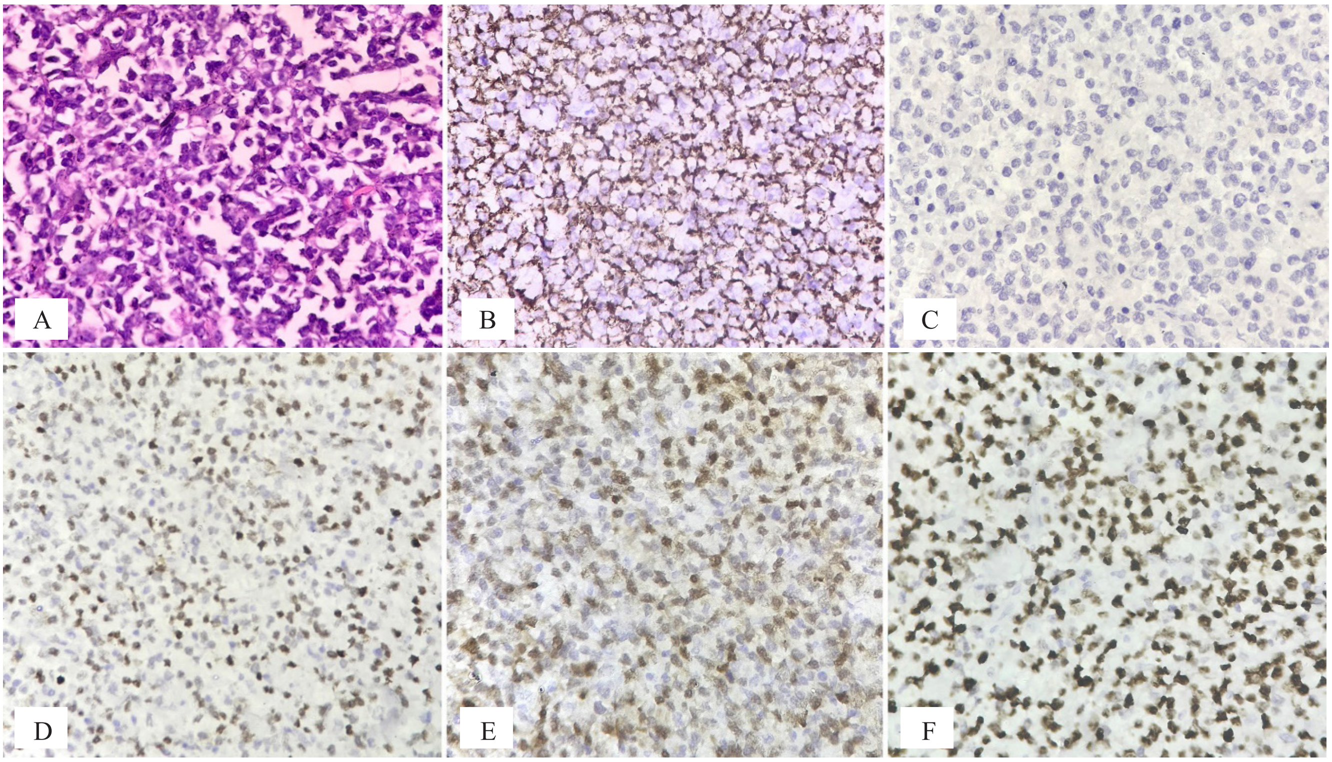

Figure 1 A: *Diffuse large B cell Lymphoma, non GCB type (H&E stain, x400). Immunohistochemical staining showing B: CD20 membranous positivity (x400). C: CD10 negative tumor cells(x400). D: BCL6 nuclear positivity(x400). E: MUM1 nuclear positivity(x400). F: high ki-67 proliferative activity(x400)*

All four cases of HL were of classic type, as shown in Table 2. All cases showed nodal involvement, with no extranodal presentation. Immunohistochemical analysis for Epstein–Barr virus latent membrane protein (EBV-LMP) was performed in three cases of HL, with one showing positivity.

Table 1: Frequency of nodal and extranodal involvement of lymphoma (n=51)

Site		Frequency
Nodal (n=25, 49%)	Cervical	18
	Axillary	2
	Submandibular	2
	Inguinal	1
	Supraclavicular	1
	Intraparotid	1
Extranodal (n=26, 51%)	GIT	8
	Tonsils	4
	Pharynx	2
	Epigastric mass	2
	Dura	1
	Hard palate	1
	Larynx	1
	Parotid	1
	Thyroid	1
	Mediastinum	1
	Testis	1
	Iliac bone	1
	Thigh	1
	Skin	1
Total		51

Table 2: Classification of Lymphoma according to 2017 WHO classification

Type	Lineage	Subtype	Frequency
Hodgkin Lymphoma (n=4, 7.8%)	B cell	Mixed cellularity	2
		Lymphocyte rich	1
		Lymphocyte depleted	1
Non Hodgkin Lymphoma (n=47, 92.2%)	B cell (n=41, 80.4%)	DLBCL	23
		Mantle cell lymphoma	5
		Marginal zone lymphoma	5
		CLL/SLL	2
		THRLBCL	2
		Follicular lymphoma	1
		Burkitts lymphoma	1
		Plasmablastic lymphoma	1
		B-lymphoblastic leukemia/lymphoma	1
	T cell (n=6, 11.8%)	PTCL, NOS	2
		ALK negative ALCL	1
		Extranodal NK/T cell lymphoma, nasal type	1
		Primary cutaneous T cell lymphoma	1
		T-lymphoblastic leukemia/lymphoma	1
Total			51

DCBCL: Diffuse large B-cell lymphoma; CLL/SLL: Chronic lymphocytic leukemia/ Small lymphocytic lymphoma,; THRLBCL: T cell histiocyte rich large B cell lymphoma; PTCL,NOS:Peripheral T cell lymphoma, Not otherwise specified; ALK negative ALCL: ALK negative Anaplastic large cell lymphoma

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DISCUSSION

This study provides an overview of lymphoma cases diagnosed at Patan Hospital. Patients ranged in age from seven to 92 years, with a mean age of 14 years in the pediatric population (≤18 years) and 61.44 years in adults. In this study, a male preponderance was observed, with a male-to-female ratio of 2:1. These findings align with national and international data demonstrating a higher incidence of lymphoma in males compared to females.[5,6]

In the present study, extranodal presentation was observed in 51% of cases, showing a slight predominance over nodal involvement. Similar findings have been reported in studies from China, which documented extranodal involvement in 55-63.5% of cases, with gastrointestinal involvement being the most commonly affected site.[7-9] This pattern has been attributed to the high prevalence of Helicobacter pylori infection in that population. In contrast, studies from India and Nepal have shown nodal predominance.[5,10] These variations may reflect underlying geographical and epidemiological differences. However, given the small sample size and single-center design of this study, larger multicenter studies are necessary to generate more representative data from Nepal. Furthermore, we identified several rare extranodal sites, including dura, thyroid, testis, hard palate, bone, parotid, and larynx. Involvement of these uncommon sites poses significant diagnostic and therapeutic challenges.[11]

Nodal involvement was seen in 49% of cases in the present study, with cervical lymph nodes being the most commonly affected, followed by the axillary and submandibular lymph nodes. These findings are consistent with other studies in which the cervical lymph nodes were the predominant site of involvement.[5,12] This predominance is likely due to the high density of lymph nodes in the neck, constant antigenic stimulation arising from the upper aerodigestive tract, and superficial location of these nodes, which facilitates easier clinical detection compared with deeper nodal sites.[13]

Precursor lymphoid neoplasms are aggressive malignancies of immature B- or T-cell precursors, characterized by TdT positivity and occurring predominantly in children and adolescents.[14] Despite their aggressive nature, these neoplasms are highly chemosensitive, with B-lineage disease showing a more favorable prognosis, particularly in younger patients.[14] The present study demonstrated a low frequency of precursor lymphoid neoplasms, accounting for 3.92% of lymphoma cases, with the majority being mature lymphoid neoplasms. Only two cases of precursor lymphoid neoplasms were identified, one each of B-lymphoblastic and T-lymphoblastic leukemia/lymphoma, both occurring in 16-year-old patients. Comparable low frequencies of precursor lymphoid neoplasms have been reported in other studies, ranging from 2.1- 3.8% of cases.[1,9]

In this study, NHL and HL were diagnosed in 92.2% and 7.8% cases, respectively, yielding an NHL to HL ratio of 11.8:1. The predominance of NHL has also been reported in other studies, although the ratios were lower such as 2.7:1 in the study by Hombegowda et al.[15], and 4.5:1 in the study by Shanmugashundarame et al.[10] Among the NHL cases, 87.2% were B-cell NHL (B-NHL) and 12.8% were T-cell NHL (T-NHL). This predominance of B-cell NHL is seen in national and international studies as well.[1,5,9,10]

Within the NHL group, DLBCL was the most common subtype, accounting for 56.1% of the cases. DLBCL is recognized as the most common type of lymphoid neoplasms globally, and the proportion observed in our study is consistent with the figures reported across various Asian and Western studies.[6,9,16]

DLBCL subgroups defined by cell of origin show differential responses to chemotherapy and targeted agents.[17] Gene expression profiling (GEP) is the gold standard for this classification and subcategorizes DLBCL into GCB type and ABC type. But GEP is largely unavailable in routine practice. IHC, which is based on protein expression, serves as a surrogate tool to divide DLBCL into GCB and non-GCB subtypes and provide similar prognostic information. The Hans algorithm is the most widely used method, employing IHC markers CD10, BCL6, and MUM1 to distinguish GCB from non-GCB subtypes, with a 72-86% concordance with GEP.[18] This distinction carries important therapeutic and prognostic implications, as patients with GCB-DLBCL typically respond well to standard R-CHOP therapy, whereas ABC-DLBCL shows inferior responses and often requires additional targeted agents such as lenalidomide, bortezomib, or ibrutinib.[19] Moreover, patients with GCB subtype have significantly better outcomes and longer progression-free survival than those with non-GCB disease.[11,20]

In this study, 65.2% were classified under GCB, and 34.8% were classified under the non-GCB type according to the Hans algorithm. The results are consistent with studies done by Ananthtamurthy A. et al and Dwivedi A et al.[12,21] However, according to Shiozawa et al., GCB subtype of DLBCL is less frequent in Asia than in the West.[22] Differences in the proportions of lymphoma subtypes between countries may reflect variations in environmental and genetic factors that influence lymphoma pathogenesis.[12]

The 2017 WHO classification of hematopoietic tumors recognized a prognostic subgroup of DLBCL, termed double-expressor DLBCL, characterized by co-expression of c-MYC and BCL-2 on immunohistochemistry.[23] It is associated with a poorer prognosis compared with non-double-expressor DLBCL and requires more aggressive therapy.[23] In the present study, MYC and BCL-2 immunohistochemistry were performed in 15 of 23 DLBCL cases, of which six demonstrated double-expressor status.

MZL and MCL were the second most common B-NHL in the present study, each with five cases. Among MZL, the extranodal type was predominant. Extranodal MZL,

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particularly of MALT type, is known to have a more favorable prognosis compared with nodal marginal zone lymphoma, with several studies reporting lower 5-year overall survival and failure-free survival rates in nodal cases.[24] Of the five cases of MCL observed in this study, two were identified as the blastoid variant. The blastoid variant of MCL is an uncommon subtype and is characterized by a more aggressive clinical course and a significantly poorer prognosis compared to classical MCL.[25]

There were two cases each of CLL/SLL and THRLBCL, and one case each of follicular lymphoma, Burkitt lymphoma and plasmablastic lymphoma. Although the literature identifies follicular lymphoma as one of the more common subtypes of NHL, it was rarely observed in the present study.[26] This may be attributable to the limited sample size, therefore further studies in larger cohorts are essential.

In the present study, T-NHL accounted for 12.8% of cases. The frequency of T-NHL varies geographically, with higher rates in Asian countries than in Western countries.[9] Peripheral TCL was the most common subtype of T-NHL in the present study, similar to that reported by Shanmugasundaram et al. and Hombegowda et al.[10,15] However, studies from China, Hong Kong, and Korea report extranodal T-cell lymphomas as the predominant subtype, likely due to their strong association with EBV, which is highly prevalent in these regions.[9]

In this study, HL accounted for 7.84% of lymphoma cases, all of which were of the classical type. Mixed cellularity was the most common subtype, followed by the lymphocyte-rich and lymphocyte-depleted variants. Other studies have also reported mixed cellularity to be the most common subtype of HL.[9,10] All cases in this study showed nodal involvement, with no extranodal presentation. Extranodal HL is very rare, constituting less than 1% of cases.[27] Immunohistochemical analysis for EBV-LMP was performed in three cases, with one showing positivity. EBV-positive HL has been associated with poorer overall and disease-specific survival, particularly in older adults, and ongoing studies are evaluating EBV latent antigens as potential targets for immunotherapy.[28,29]

LIMITATIONS:

This study is limited by its small sample size and single-center design. The lack of molecular and cytogenetic testing and reliance on IHC instead of gene expression profiling may reduce diagnostic precision. Future multicenter, prospective studies with larger cohorts and incorporation of molecular techniques are needed to provide more representative data on lymphoma subtypes in the Nepalese population.

CONCLUSIONS:

This study provides a comprehensive overview of the spectrum of lymphoma cases diagnosed at Patan Hospital over two years. NHL predominated, accounting for over 90% of cases, with B-cell lymphomas, particularly DLBCL, being the most common subtype. T-cell lymphomas and HL were less frequent. Extranodal involvement was seen in over half of the cases, with the gastrointestinal tract being the most commonly affected site. Immunohistochemistry proved invaluable for accurate subclassification, guiding prognosis, and informing potential targeted therapies. This study emphasizes the importance of histopathological and immunophenotypic evaluation of lymphoma cases and provides baseline epidemiological data for future studies in the region.

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Conflict of interest: None

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