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Original Article

Immunohistochemical profile and distribution pattern of Non-Hodgkin and Hodgkin lymphoma - an institutional study

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Diffuse Large B-Cell Lymphoma, Hodgkin Lymphoma, Immunohistochemistry, Non-Hodgkin Lymphoma

ABSTRACT

Background: Lymphomas are a group of heterogeneous lymphoproliferative malignancies with a distinctive behavior and treatment responses. Immunohistochemistry guides the accurate subtyping, diagnosis, and prognostication of lymphomas. The objective of this study was to observe subtypes of lymphoma and their distribution and frequency by age and gender.

Materials and Methods: A two-year retrospective study (2020-2022) was conducted in the Department of Pathology, Vydehi Institute of Medical Sciences and Research Centre, India. Cases were subcategorized using panels of Immunohistochemistry.

Results: Of 100 lymphoma cases, 73 were Non-Hodgkin lymphoma and 27 Hodgkin lymphoma. Males outnumbered females in both groups. Among non-hodgkin lymphoma, Diffuse large B-cell lymphoma, not otherwise specified comprised 56%, with 29% being anaplastic variant, followed by small lymphocytic lymphoma. In Hodgkin lymphoma, 25 cases were Classical hodgkin lymphoma, and 2 were Nodular lymphocyte predominant hodgkin lymphoma. Nodal involvement was more frequent, 73.6% in Non-Hodgkin lymphoma and 85.1% in Hodgkin lymphoma, with the cervical lymph node most commonly affected. Extranodal disease accounted for 27.3% of Non-Hodgkin lymphoma and 14.8% of Hodgkin lymphoma.

Conclusions: Non-Hodgkin Lymphomas predominated over Hodgkin Lymphoma, with Diffuse large B-cell Lymphoma-NOS being the most common subtype. Immunohistochemistry remains essential for precise lymphoma subclassification, ensuring accurate diagnosis.

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INTRODUCTION

The lymphomas are a heterogeneous group of lympho-proliferative malignancies, with distinct causes and showing distinctive patterns of behavior and responses to treatment. There has been a rising incidence of lymphoma.[1] The incidence varies among different countries as well as within various regions of the country. The age-adjusted incidence of Non-Hodgkin Lymphoma (NHL) in India is 5/100000. NHL accounts for 5.1% of all malignancies, while Hodgkin Lymphoma (HL) constitutes 9.5% of cancers in men and 5.5% in women.[1]

The World Health Organization tumor classification distinguishes lymphoid neoplasms derived from precursor lymphoid cells from those derived from mature lymphoid cells and further separates each group into neoplasms of B-cell or T-cell origin. Tumors of mature histiocytic and dendritic cell (HDC) origin are not derived from lymphoid cells but often involve lymphoid tissue and historically have been discussed along with mature lymphoid neoplasms.[2]

The etiology of lymphomas is largely unknown. Some of the risk factors include severe immunodeficiency, various infectious agents, familial aggregation, blood transfusion, and occupational exposure to pesticides and solvents.[3] With the advent of changes in lymphoma therapy, the accurate diagnosis of lymphoma and prognostication, wherever possible, becomes critical. Similarly, the classification of lymphomas has evolved with the incorporation of various novel immunohistochemical markers in the diagnosis and prognostication.

The panel of markers is decided based on morphologic differential diagnosis, which includes leukocyte common antigen (LCA), B-cell markers (CD20 and CD79a), T-cell markers (CD3 and CD5) and other markers like CD23, bcl-2, CD10, cyclin-D1, CD15, CD30, ALK-1, and CD138.[4]

Among HLs, Mixed cellularity (MC) is the most common subtype. Lymphocyte-rich Hodgkin lymphoma (LRCHL) is relatively infrequent, accounting for 3–5% of CHL. NLPHL is rare, accounting for 3–5% of Hodgkin lymphomas.[5]

NHL ranks as the tenth and twelfth most frequent malignancy in males and females respectively, worldwide. The subtype distribution varies geographically. Follicular lymphoma and Chronic lymphocytic leukemia are more frequent in the Western population, whereas Diffuse large B-cell lymphoma (DLBCL) is most prevalent in India.[6]

PTCL-NOS comprises a heterogeneous group of mature T-cell lymphomas that do not meet diagnostic criteria for one of the more specific mature T-cell neoplasms; as such, it is a diagnosis of exclusion.[5] PTCL-NOS remains the most commonly diagnosed subtype of T-cell lymphoma.

The study was undertaken to subtype distribution pattern of lymphoma based on World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th edition, using IHC, and also to study the distribution pattern and frequency of lymphoma by age and gender.

MATERIALS AND METHODS:

Two-year retrospective study was carried out from the year 2020 to 2022, during which 100 cases diagnosed as lymphoma in the histopathology department at Vydehi Institute of Medical Sciences and Research Centre were included in the study. The data collected was statistically analyzed using SPSS software version 21. Qualitative variables were expressed as frequencies and percentages, while quantitative variables were expressed as mean/-standard deviation. Patients of all ages and genders with both primary and extranodal lymphomas were included in the study. For histopathological and immunohistochemical studies, the tumor samples were fixed in 10% neutral buffered formalin, and after tissue processing, they were embedded in a paraffin block. Histopathological diagnosis was made on routine hematoxylin and eosin stained sections, with subsequent immunohistochemical examination using various panels of antibodies based on morphological interpretation. The antibodies utilized included CD20, CD3, CD45, CD15, CD30 CD5, CD4, CD8, CD10, CD23, BCL2, BCL6, CYCLIN-D1, KI67, TdT, CD38, CD138, MUM1, PAX5, Pan-CK and ALK.

RESULTS

During the study period, out of 100 cases of lymphomas, seventy-three cases (73%) were NHL, and twenty-seven cases (27%) were HL. Among 73 cases of NHL, 58.9% (n=43) were male and 41% (n=30) were female. Of 27 cases of HL, 59.2% (n=16) were male and 40.7% (n=11) were female (Table 1). In both groups, males outnumbered females (male-to-female ratio being 1.4:1). Of 27 cases, 13 were in the age group of 0-30 years and 14 were in the age group of 30-60 years. Among patients with NHL, 57 were in the age group of 40-80 years and 16 were in the age group of 20-40yrs. In NHL, nodal involvement was seen in 73.6% (n=53) and extranodal disease was seen in 27.3% (n=20). In HL, nodal involvement constituted 85.1% (n=23), and extranodal involvement was seen in 14.8% (n=4) (Table 2).

Among 73 cases of NHL, B-cell Non-Hodgkin Lymphoma (B-NHL) formed 95.8% (n=70) cases, while T-cell Non-Hodgkin Lymphoma (T-NHL) formed 4.1% (n=3) cases. (fig.1A) DLBCL-NOS (Diffuse Large B-cell Lymphoma-Not otherwise specified) was the most common B-NHL, constituting 56% (n=41) of all the NHLs. The second most common was Follicular lymphoma 15.6% (n=11). (fig.1B) Out of 41 cases of DLBCL-NOS, 28 (68.2%) cases presented with nodal involvement and 13 (31.7%) cases showed extranodal presentation. Among 27 cases of HL, 25 cases (92.5%) were of Classical Hodgkin lymphoma (CHL), 2 cases (7.4%) were of Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL). 26 cases of HL presented with nodal involvement, and 1 case presented with bone as extranodal involvement.

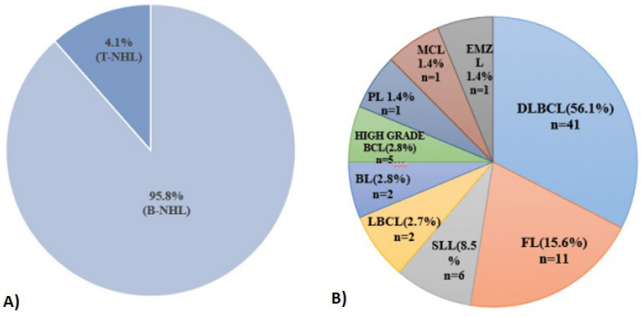

Figure 1: *A) Distribution of Non-Hodgkin Lymphoma; B) Distribution of B-cell Non-Hodgkin Lymphoma*

Table 1: Frequency of Lymphoma classified by gender

Diagnosis	Male	Female
Classical Hodgkin lymphoma	14(56%)	11(40%)
Nodular lymphocyte predominant Hodgkin lymphoma	2(100%)	0(0%)
Small lymphocytic lymphoma	4(66.6%)	2(33.3%)
Diffuse large B-cell lymphona	25(60.9%)	16(39.0%)
High grade and large B-cell lymphoma	4(75%)	1(30%)
Low grade B-cell lymphoma	1(50%)	1(50%)
Follicular lymphoma	4(36.3%)	7(63.6%)
Mantle cell lymphoma	1(100%)	0(0%)
Plasmablastic lymphoma	1(100%)	0(0%)
Extranodal marginal zone lymphoma	1(100%)	0(0%)
Burkitt lymphoma	2(100%)	0(0%)
Peripheral T-cell lymphoma	0(0%)	3(100%)
Total cases	**59(59%)**	**41(41%)**

Table 2: Nodal and extranodal distribution of Lymphoma

Diagnosis	Number of cases	Nodal n (%)	Extranodal n (%)
Classical Hodgkin lymphoma	25	21 (84%)	4 (16%)
Nodular lymphocyte predominant Hodgkin lymphoma	2	2 (100%)	0 (0%)
Small lymphocytic lymphoma	6	6 (100%)	0 (0%)
Diffuse large B-cell lymphoma	41	28 (68.2%)	13 (31.7%)
High-grade and large B-cell lymphoma	5	2 (40%)	3 (60%)
Low grade B-cell lymphoma	2	2 (100%)	0 (0%)
Follicular lymphoma	11	10 (90.9%)	1 (9.09%)
Mantle cell lymphoma	1	1 (100%)	0 (0%)
Plasmablastic lymphoma	1	1 (100%)	0 (0%)
Extranodal marginal zone lymphoma	1	0 (0%)	1 (100%)
Burkitt lymphoma	2	1 (50%)	1 (50%)
Peripheral T-cell lymphoma	3	2 (66.6%)	1 (33.3%)
Total cases	**100**	**76 (76%)**	**24 (24%)**

Immunohistochemistry analysis:

Neoplastic cells in CHL cases were positive for CD30 and PAX5, with background cells showing positivity for CD3. Neoplastic cells in NLPHL were positive for CD20 and PAX5. All Small lymphocytic lymphoma cases are positive for CD20, CD5, CD23, and BCL2. Among 41 cases of DLBCL, 6 (14.6%) were CD10 positive, 19 cases (46%) were positive for BCL2, and 15 cases (36.5%) were positive for BCL6. Anaplastic variant of DLBCL accounted for 34% (12 cases) of all DLBCL cases, out of which 10 cases were positive for CD30, 7 showed CD20 positivity, 3 were positive for ALK1, and 5 demonstrated MUM1 positivity. All 11 cases of Follicular lymphoma were positive for CD20 and BCL2. Burkitt lymphoma cases were positive for CD45, CD20, and CD10 with an almost 100% Ki67. PTCL were positive for CD45, CD3 and CD5 (Table 3).

Table 3: Immunohistochemical distribution of lymphomas

Diagnosis	Common Positive Markers	Less Common Positive Markers	Negative Markers
Classical Hodgkin Lymphoma	CD45, CD30, PAX5	CD15	CD20
Nodular Lymphocyte Predominant Hodgkin Lymphoma	CD45, CD20, CD30, PAX5		CD15, CD10
Small Lymphocytic Lymphoma	CD20, CD5, CD23 BCL2		CylinD1, CD10, BCL6
Diffuse Large B-Cell Lymphoma – Not Otherwise Specified	BCL2, CD10, CD30	ALK1, MUM1, BCL6	CD3, CD5
High Grade & Low-grade B-cell Lymphoma	CD45, CD20, CD3, BCL6		BCL2, MUM1
Low-grade B-Lymphoma	CD45, CD20	CD10, BCL2	CD23, CD5
Follicular Lymphoma	CD20, BCL2, BCL6, CD10	CD5	CD3
Mantle Cell Lymphoma	CD45, CD3, CD5, CD20, CyclinD1		CD15, CD30, CD10, MUM1, BCL6
Plasmablastic Lymphoma	CD138, MUM1, CD45		CD20, CD10, BCL6, BCL2
Extranodal Marginal Zone Lymphoma	CD45, CD3, CD20, CD21, CD35		CD10, CD5, BCL6
Burkitt Lymphoma	CD45, CD20, CD10	BCL6	CD5, TdT
Peripheral T-cell Lymphoma	CD45, CD3, CD5, CD7	BCL2, BCL6, MUM1	CD20, CD30, CD10

DISCUSSION

In developing countries, the prevalence and incidence of lymphoma, as well as the distribution of lymphoma subtypes, may vary. In the current study, HL and NHL were diagnosed in 27% and 73% of cases, respectively, which is consistent with the results of a survey done by Shanmugasundaram et al. in Coimbatore, Tamilnadu, India.[1]

The present study showed male preponderance. The median age of presentation in the present study was 40 years. The ratio of NHL to HL was 2.7:1 in the present study, which is lesser as compared to the study done by Shanmugasundaram et al,1 which reported a ratio of NHL to HL to be 4.5:1. DLBCL-NOS was the most common type of aggressive NHL and constituted 30%–58%.

This was observed in the present study as well as studies by several other authors worldwide. In line with most other studies in India, the most common mode of presentation in the present study was lymphadenopathy, constituting 73%. The most commonly involved lymph node group was the cervical lymph node, which is in line with the study done by Hansa M. Goswami et al.[2], followed by mediastinal and mesenteric lymph nodes.

Extranodal lymphomas account for approximately one-third of Non-Hodgkin lymphomas (NHL) and these originate from sites other than lymph nodes, spleen, or the bone marrow. Extranodal lymphomas can arise in almost every organ. In the literature, it is shown that the gastrointestinal (GI) tract, skin, bone, and brain are the most common sites. Ann Arbor staging system considers tonsils and Waldeyer's ring as lymphatic localizations, but there is controversy regarding their designation as extranodal sites. If they were included in the extranodal lymphoma category, the head and neck would be the second most frequent site.[11] In the present study, extranodal lymphomas were predominantly seen in the DLBCL subtype, constituting 39%. The gastrointestinal tract was the most common site of extranodal lymphomas, consistent with the study done by Vannata B Zucca et al.[9]

B-cell Non-Hodgkin lymphoma:

Age and sex are two known demographic risk factors for NHL, according to Alyahya et al.[12] The present study also reports male preponderance as observed in the study done by Adomako J et al.[8] Moreover, Horesh and Horowitz et al.[13] linked the number of pregnancies and live births, as well as sex hormones such as estrogen, to the reduction in the risk of NHL in females. The ratio between B-cell and T-cell NHL is 23.3:1. DLBCL-NOS, constitutes 25-35% of adult NHLs in developed countries, and a higher percentage in developing countries. In the present study, among 41 cases of DLBCL-NOS, 12 cases (29.2%) were Anaplastic variant. DLBCL-NOS was followed in frequency by FL, constituting 15.06%, which is concordant with the study done by Hansa M. Goswami et al.[2] Out of 11 cases of FL, 10 cases showed nodal involvement and 1 case showed extranodal involvement (left tonsil).

Morphology:

Anaplastic variant of DLBCL: This variant is characterized by large to very large cells with bizarre pleomorphic nuclei that may resemble Hodgkin/ Reed-Sternberg cells, and may resemble the neoplastic cells of anaplastic large cell lymphoma. The neoplastic cells may exhibit a sinusoidal and/or cohesive growth pattern and may mimic undifferentiated carcinoma as well. The anaplastic variant is biologically and clinically unrelated to anaplastic large cell lymphoma, which is often of cytotoxic T-cell derivation, and unrelated to ALK-positive large B-cell lymphoma, which lacks expression of CD20 and CD30.[7] (fig.2) Characteristic feature of the anaplastic DLBCL is the diffuse expression of the CD30 immunohistochemical (IHC) marker.[10]

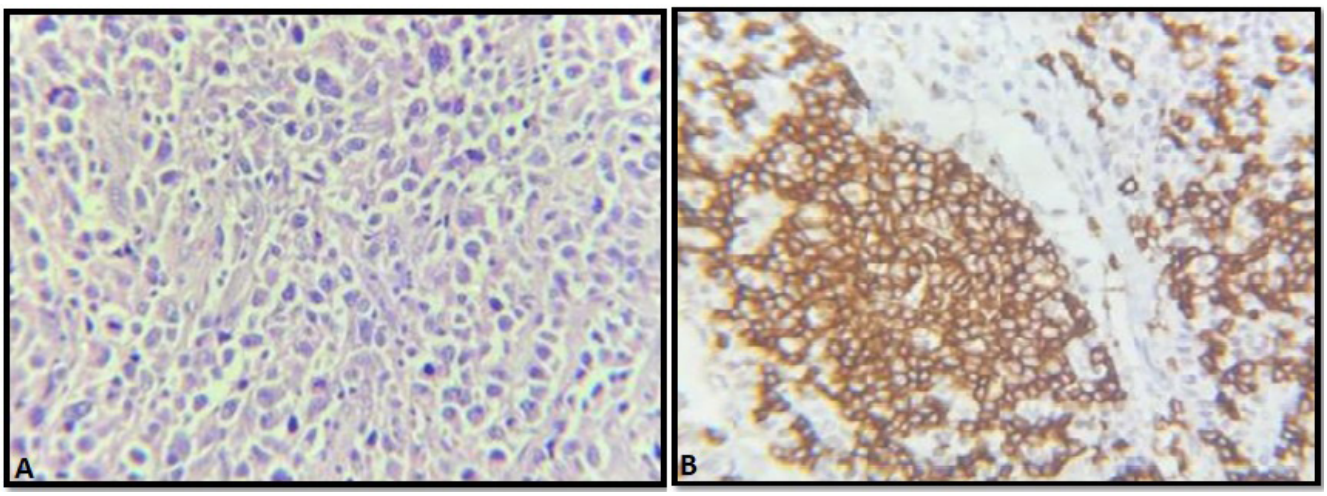

Figure 2: *(A) Anaplatic variant of Diffuse Large B-cell Lymphoma (H&E; 40x); (B) Anaplastic variant of Diffuse Large B-cell Lymphoma expressing CD20 (IHC; 40x).*

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T-cell Non-Hodgkin Lymphoma: T-NHL formed 4% (n=3) of cases, which is similar to other Indian studies. PTCL was the most common subtype of T-NHL in the present study, similar to that reported by Shanmugasundaram et al.[1] CD7 is the earliest Tcell lineage marker to be expressed, followed by CD2, CD5, and CD3 in the bone marrow. In the present study, neoplastic cells in PTCL were positive for CD45, CD3, CD5, BCL2, BCL6, and MUM1.

Hodgkin lymphoma: CHL is the more common of the two Hodgkin lymphoma entities and can be further divided into four histological subtypes: Lymphocyte-rich CHL (LRCHL), Nodular sclerosis CHL, Mixed cellularity CHL, and Lymphocyte-depleted CHL.[3] In the present study, out of 27 cases of HL, 25 cases (92.5%) were CHL and 2 cases (7.4%) were NLPHL. Hodgkin lymphoma showed bimodal presentation with a median age of 30 years.

Morphologically, there are presence of Reed-Sternberg cells in the background of a reactive mixed population lymphocytes, histiocytes, plasma cells, eosinophils, neutrophils, and fibroblasts.3 The Reed Sternberg cells in all forms of CHL share similar immunophenotypic features, with reduced expression of most B-cell antigens (CD20, CD79a, PAX5) and positive staining for CD30 and CD15 in most cases.[5] (fig.3)

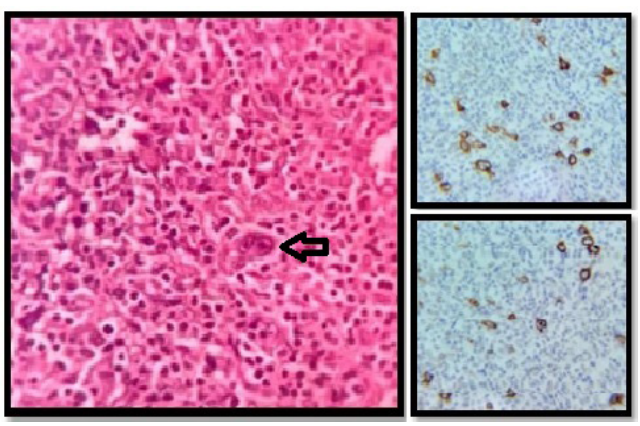

Figure 3: *Hodgkin Lymphoma. (A) Reed-Sternberg cell (arrow) [H&E, 100x]; B&C) Neoplastic cells showing CD30 (B) and CD15 (C) positivity [IHC; 100x].*

CONCLUSIONS

In the present study, NHLs were found to be more predominant than HLs. In both the groups, males outnumbered females (male-to-female ratio being 1.4:1). DLBCL was the common subtype of NHL, of which 29% of cases were Anaplastic variant. DLBCL was followed by FL (15.6%) and SLL (8.2%). HL exhibited bimodal presentation, with 36 years being the median age among NHL cases. In both HL and NHL, nodal involvement was more common than extranodal sites. Of 24 cases of HL, 23 cases presented with nodal involvement, and 1 case presented with bone as extranodal involvement. In NHL, nodal involvement was 73.6% (n=53), and extranodal was 27.3% (n=20). The cervical lymph node was the most frequently involved, and the gastrointestinal tract being the most common site of extranodal involvement.

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