e-ISSN: 0975-9506, p-ISSN: 2961-6093

Available online on www.ijpqa.com doi: 10.25258/ijpqa.16.12.116

International Journal of Pharmaceutical Quality Assurance 2025; 16(12); 716-722

Original Research Article

Comparative Analysis of Lymphoma Variants Based on Histological Features and IHC Profiling

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Received: 02-11-2025 / Revised: 21-11-2025 / Accepted: 25-12-2025
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Conflict of interest: Nil

Abstract:

Background: Lymphomas represent a heterogeneous group of lymphoid malignancies with diverse histological and immunophenotypic profiles. Accurate classification is essential for diagnosis, prognostication, and therapy.

Aim: To perform a comparative analysis of lymphoma variants based on histological features and immunohistochemical (IHC) profiling.

Methodology: This hospital-based cross-sectional study included 80 lymph node biopsy cases diagnosed as lymphoma at the Department of Pathology, Phulo Jhano Medical College, Dumka, Jharkhand, over 8 months. Specimens were processed for histology using Hematoxylin and Eosin staining and further analyzed with an IHC panel including CD3, CD15, CD20, CD30, LCA, Bcl-2, and Pancytokeratin. Lymphomas were classified according to the 2017 WHO criteria, and data were analyzed descriptively using SPSS v27.

Results: Non-Hodgkin lymphomas (NHL) comprised 71.3% of cases, with Diffuse Large B-Cell Lymphoma (DLBCL) being predominant (35%). Hodgkin lymphomas (HL) represented 28.7%, mainly classical HL. IHC profiling showed CD20 and LCA positivity in NHL, while CD15 and CD30 were characteristic of HL. A male predominance (62.5%) and peak incidence in the 41–60 years age group were observed.

Conclusion: Histological assessment combined with IHC profiling is critical for accurate subclassification of lymphoma variants, guiding targeted therapy and prognostication.

Keywords: Lymphoma, Histology, Immunohistochemistry, Diffuse Large B-Cell Lymphoma, Hodgkin Lymphoma.

Introduction

The lymph node serves as one of the major body parts that make up the immune system because it helps start and manage immune responses according to research [1]. The structure of lymph nodes divides into three parts which include the cortex the paracortex and the medulla. Normal immune responses cause lymph node growth because the immune system needs to produce additional cells which result in permanent lymph node growth according to research findings [2]. Physicians must evaluate this natural body growth because it creates confusion with medical conditions which need clinical assessment for precise identification.

Lymphadenopathy is a common clinical problem which doctors see in their regular medical work because it affects people from every age group. Doctors use biopsies to identify the reason behind nodal enlargement which can either be caused by neoplastic conditions or non-neoplastic conditions according to [3]. The etiological spectrum of lymphadenopathy is broad and includes infections, autoimmune conditions, storage disorders, primary lymphoid malignancies, and metastatic malignancies. The histopathological examination of lymph node biopsies serves as an essential method for identifying between reactive processes and malignant neoplasms.

Lymph node lesions form a wide range of spectrum from benign reactive changes to lymphoma and metastatic deposits [4]. Reactive lymphadenopathy shows three distinct patterns which include follicular hyperplasia, paracortical hyperplasia, and sinus histiocytosis to demonstrate its response to immunologic stimuli. Lymphomas emerge as clonal cell expansions of lymphoid cells which display both architectural structure loss and atypical cytological characteristics and unusual immunophenotypic patterns. The differential diagnosis process becomes more challenging because non-lymphoid cancers create additional metastatic deposits. The presence of similar physical characteristics between reactive and neoplastic conditions requires additional methods to achieve accurate identification.

Clinicians divide lymphadenopathy into two distinct categories which include generalized lymphadenopathy and localized lymphadenopathy. Generalized lymphadenopathy occurs in many systemic diseases which include infections and autoimmune disorders and hematological cancers while localized lymphadenopathy occurs more frequently with local infections or malignancies [5]. The clinical distinction establishes an initial assessment framework yet it does not provide sufficient information to reach a final diagnostic conclusion. The histological examination serves as essential method for determining specific pathological condition.

The pathologists first need to identify separate disease entities which exist as distinct medical conditions before researchers can create improved treatment methods. The most common form of reactive lymphadenopathy lacks an effective treatment yet any diagnosis which is not specific provides value because doctors need to rule out cancer and treatable conditions according to [6]. The accurate identification of specific lymphoma types becomes vital when doctors detect a lymphoid malignancy because different treatment methods and patient survival prospects require different lymphoma types. The development of lymphoma classification systems has progressed from using morphological characteristics to modern integrated systems that combine immunophenotypic and molecular knowledge for enhanced diagnostic accuracy and clinical usefulness.

Lymphomas divide into two main categories which are Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) and each category contains several histological subtypes that exhibit different biological characteristics [7]. The first diagnostic method for Hodgkin lymphoma relies on histological examination which studies architectural patterns and cytological features and identifies Reed-Sternberg cells through their specialized cells. The diagnostic process requires more than morphological evaluation because non-Hodgkin lymphomas exhibit features that overlap between their various subtypes.

The diagnostic process has received essential support from immunohistochemistry testing which functions as an important supplementary tool. Immunohistochemistry assists in dividing lymphomas into distinct subtypes which have both treatment value and prognostic significance [8]. The IHC test identifies cell lineage and differentiation status and abnormal antigen expression through its specific antibodies against B-cell and T-cell lineage markers. The immunophenotypic profiling process establishes the neoplastic cells' lymphoid origin while it also identifies different lymphoma types that share similar structural characteristics.

The identification of B-cell markers such as CD20 and T-cell markers such as CD3 helps establish lineage assignment while CD10 BCL6 MUM1 Cyclin D1 CD15 and CD30 serve as additional markers for ongoing subclassification [9]. The modern method for diagnosing lymphoma relies on histomorphology and IHC findings. The classification process establishes risk levels that help determine suitable treatment options which encompass both monoclonal antibody therapies and new immunotherapy approaches.

The comparative analysis of lymphoma variants through their histological characteristics and IHC profiling establishes essential clinical and pathology significance. The analysis enables researchers to study different patterns of morphology and immunophenotyping and their relationship with clinical symptoms. The process assists with diagnosing difficulties which involve grey zone lymphomas and cases that show unusual marker expressions. The combination of histopathology and IHC testing serves as the most effective and dependable method for diagnosing lymphoma in areas that lack access to advanced molecular testing.

The various lymphoma patterns which exist in different populations demonstrate the need for research studies which focus on specific regional areas. The different epidemiological patterns between lymph node biopsy results and patient demographics and disease symptoms make it essential to conduct systematic evaluations of lymph node biopsy studies. The structured comparative study enables researchers to record occurrence rates and tissue analysis results and antibody testing outcomes for multiple lymphoma types which helps doctors make correct diagnoses and treat their patients more effectively.

Lymphadenopathy constitutes a common medical condition which presents diagnostic difficulties for doctors. The primary focus should lie on verifying which malignant lymphomas exist and determining their exact subtype. The diagnosis process requires histological examination to provide structural and cytological information while the immunohistochemistry process helps to refine and confirm subtype identification. The complete comparison of lymphoma types through histological analysis and IHC testing establishes the foundation for precise diagnosis and prognosis assessment and treatment selection. The current study uses the integrated approach which serves as the fundamental method for modern hematopathology research".

Methodology

Study Design: The present study was designed as a hospital-based cross-sectional observational study aimed at conducting a comparative analysis of various lymphoma variants based on histological features and immunohistochemical (IHC) profiling.

Study Area: The study was carried out in the Department of Pathology, Phulo Jhano Medical College, Dumka, Jharkhand, India.

Study Duration: The study was conducted over a period of 8 months from March 2025 to October 2025.

Study Participants

Inclusion Criteria

- Excision biopsies of lymph nodes received in the Department of Pathology during the study period.
- Cases with a confirmed histopathological diagnosis of lymphoma.
- Adequately preserved formalin-fixed, paraffin-embedded tissue specimens suitable for histological and IHC analysis.

Exclusion Criteria

- Cases with inconclusive diagnosis due to inadequate or poorly preserved tissue material.
- Needle biopsies and cytology specimens lacking sufficient architectural details.
- Cases with incomplete clinical or laboratory data.

Sample Size: A total of 80 cases fulfilling the inclusion criteria were included in the study.

Procedure: All lymph node specimens received in the Department of Pathology were fixed in 10% neutral buffered formalin immediately after excision. After adequate fixation, the tissues were processed routinely, embedded in paraffin, and sectioned at 4–5 µm thickness. The sections were stained with Hematoxylin and Eosin (H&E) for detailed histomorphological examination. Microscopic evaluation was carried out to assess architectural patterns, cytological characteristics, presence of Reed–Sternberg cells, nodularity, fibrosis, necrosis, and other relevant features suggestive of specific lymphoma subtypes.

Based on the initial histomorphological assessment, immunohistochemistry was performed wherever required for confirmation and subtyping. IHC studies were conducted on 5 µm paraffin sections using appropriate monoclonal antibodies. The antibody panel included CD3, CD15, CD20, CD30, Leukocyte Common Antigen (LCA), Pancytokeratin, and Bcl-2. All cases diagnosed as non-Hodgkin lymphoma were subjected to B-cell and T-cell markers for accurate immunophenotypic classification. Similarly, most cases of Hodgkin lymphoma underwent immunophenotyping using CD45, CD15, and CD30 to confirm the diagnosis. In cases where there was diagnostic ambiguity, Pancytokeratin was employed to rule out metastatic epithelial malignancies.

The lymphomas were classified according to the 2017 World Health Organization (WHO) classification of hematolymphoid malignancies. Histological and immunophenotypic findings were correlated to establish the final diagnosis and to facilitate comparative analysis among different lymphoma variants.

Statistical Analysis: The collected data were entered into Microsoft Excel and subsequently analyzed using Statistical Package for the Social Sciences (SPSS) version 27.0. Descriptive statistics such as frequencies and percentages were used to summarize categorical variables including age distribution, gender distribution, lymphoma subtype, and immunohistochemical marker expression. Comparative analysis between different lymphoma variants based on histological features and IHC profiles was performed using appropriate statistical tests such as the Chi-square test. A p-value of less than 0.05 was considered statistically significant".

Result

Table 1 shows the age and gender distribution of lymphoma cases among the 80 study participants. The highest proportion of cases was observed in the 41–60 years age group, comprising 26 patients (32.5%), indicating that middle-aged individuals were most commonly affected. This was followed by the 21–40 years and >60 years age groups, each accounting for 22 cases (27.5%). The least number of cases was recorded in the 0–20 years group, with 10 patients (12.5%). Gender-wise distribution revealed a clear male predominance, with 50 males (62.5%) compared to 30 females (37.5%). In every age category, males outnumbered females, particularly in the 41–60 years group (16 males vs. 10 females). Overall, the findings suggest that lymphoma was more prevalent among males and most commonly occurred in the middle-aged population within the study sample.

Table 1: Age and Gender Distribution of Lymphoma Cases (N = 80)

Age Group (Years)	Male (n)	Female (n)	Total (n)	Percentage (%)
0–20	6	4	10	12.5
21–40	14	8	22	27.5
41–60	16	10	26	32.5
>60	14	8	22	27.5
Total	**50**	**30**	**80**	**100**

Table 2 shows the distribution of lymphoma variants based on histological classification among 80 patients. The most common subtype was Diffuse Large B-Cell Lymphoma (DLBCL), accounting for 28 cases (35%), indicating its predominance in the study population. This was followed by Classical Hodgkin Lymphoma with 18 cases (22.5%), representing the second most frequent variant. Follicular Lymphoma constituted 10 cases (12.5%), while T-Cell Lymphoma was observed in 8 patients (10%). Small Lymphocytic Lymphoma accounted for 6 cases (7.5%). Nodular Lymphocyte Predominant Hodgkin Lymphoma and Mantle Cell Lymphoma were the least common variants, each comprising 5 cases (6.3%). Overall, non-Hodgkin lymphomas formed the majority of cases, with DLBCL being the dominant histological subtype in this cohort.

Table 2: Distribution of Lymphoma Variants Based on Histological Classification (N = 80)

Lymphoma Variant	Frequency (n)	Percentage (%)
Diffuse Large B-Cell Lymphoma (DLBCL)	28	35
Follicular Lymphoma	10	12.5
Small Lymphocytic Lymphoma	6	7.5
T-Cell Lymphoma	8	10
Classical Hodgkin Lymphoma	18	22.5
Nodular Lymphocyte Predominant HL	5	6.3
Mantle Cell Lymphoma	5	6.3
Total	**80**	**100**

Table 3 shows the distribution of Non-Hodgkin and Hodgkin lymphomas among the 80 study participants. Out of the total cases, 57 patients (71.3%) were diagnosed with non-Hodgkin lymphoma, while 23 patients (28.7%) had Hodgkin lymphoma. This indicates that non-Hodgkin lymphoma constituted more than two-thirds of the cases in the present study, making it the predominant lymphoma subtype, whereas Hodgkin lymphoma accounted for less than one-third of the total cases. Overall, the findings demonstrate a substantially higher occurrence of non-Hodgkin lymphoma compared to Hodgkin lymphoma in the study population.

Table 3: Distribution of Non-Hodgkin and Hodgkin Lymphomas (N = 80)

Category	Frequency (n)	Percentage (%)
Non-Hodgkin Lymphoma	57	71.3
Hodgkin Lymphoma	23	28.7
Total	**80**	**100**

Table 4 shows the distribution of immunohistochemical (IHC) marker expression in 80 lymphoma cases. Among the markers studied, LCA demonstrated the highest positivity, being expressed in 75 cases (93.8%), confirming its strong association with lymphoid origin. CD20 was positive in 48 cases (60%), indicating a predominance of B-cell lymphomas in the study population. CD3 positivity was observed in 15 cases (18.8%), reflecting a smaller proportion of T-cell lymphomas. CD15 and CD30 were positive in 18 (22.5%) and 20 (25%) cases respectively, markers commonly associated with certain subtypes such as classical Hodgkin lymphoma. Bcl-2 expression was noted in 30 cases (37.5%), suggesting its role in tumor cell survival and anti-apoptotic activity in a subset of lymphomas. Pancytokeratin showed minimal positivity in only 2 cases (2.5%), helping to exclude epithelial malignancies and confirming the lymphoid nature of most tumors. Overall, the immunophenotypic profile indicates a predominance of B-cell lineage lymphomas with high LCA expression and variable expression of other lineage-specific and diagnostic markers.

Table 4: Immunohistochemical Marker Expression in Lymphoma Cases (N = 80)

IHC Marker	Positive (n)	Negative (n)	Percentage Positive (%)
CD20	48	32	60
CD3	15	65	18.8
CD15	18	62	22.5
CD30	20	60	25
LCA	75	5	93.8
Bcl-2	30	50	37.5
Pancytokeratin	2	78	2.5

Table 5 shows the association between lymphoma category and key immunohistochemical (IHC) markers among 80 cases. Out of 57 cases of Non-Hodgkin Lymphoma (NHL), CD20 positivity was observed in 45 cases, indicating a strong predominance of B-cell lineage in NHL, whereas only 3 cases of Hodgkin Lymphoma (HL) showed CD20 positivity. CD3 positivity was seen in 14 NHL cases and 1 HL case, reflecting a smaller proportion of T-cell–derived lymphomas. In contrast, CD15 and CD30, which are characteristically associated with classical HL, were highly expressed in HL cases (16 each) compared to minimal expression in NHL cases (2 and 4 respectively). Leukocyte Common Antigen (LCA) was positive in all 57 NHL cases and in 18 HL cases, supporting its role as a pan-leukocyte marker with stronger consistency in NHL. Overall, the table demonstrates that CD20 and LCA are predominantly associated with NHL, while CD15 and CD30 are strongly linked with HL, highlighting the diagnostic utility of IHC markers in differentiating lymphoma subtypes.

Table 5: Association Between Lymphoma Category and Key IHC Markers (N = 80)

IHC Marker	Non-Hodgkin Lymphoma Positive (n=57)	Hodgkin Lymphoma Positive (n=23)
CD20	45	3
CD3	14	1
CD15	2	16
CD30	4	16
LCA	57	18

Discussion

The researchers examined 80 lymphoma cases to study how different demographic groups and histological subtypes and immunophenotypic profiles appear in the disease. In our cohort, lymphoma predominantly affected middle-aged adults (41–60 years), followed by younger adults (21–40 years), with the lowest incidence in patients aged 0–20 years. The observed age distribution matches the findings of Roy et al. (2013) [10], who discovered that lymphomas reached their highest occurrence in South India's 41–60 years age group, while Vallabhajosyula et al. (2010) [11] found that non-Hodgkin lymphomas (NHL) occurred most often in middle-aged people. The studies by Damle et al. (2017) [12] found that younger people between 21 and 30 years old made up most of the cases, which indicates that lymphoma epidemiology shows different patterns throughout regions and populations".

The study results showed that our research group had more male participants who belonged to the middle-aged demographic. The study results from Roy et al. 2013 and Hussain et al. 2019 [13] showed that their research groups had a male-to-female ratio of approximately 1.5:1. The studies conducted by Damle et al. 2017 and Saraswat et al. 2015 [14] showed that female participants outnumbered male participants because regional and genetic factors influenced the study results. The study showed that researchers most often performed biopsies on cervical lymph nodes but they also conducted biopsies on inguinal nodes. Khanday et al. 2019 [15] demonstrated that lymphomas show a tendency to affect superficial nodal areas because these regions are both easier to access and more likely to show signs of disease at an early stage.

Non-Hodgkin lymphomas which showed histological evidence made up most of the cases while Diffuse Large B-Cell Lymphoma (DLBCL) formed the main subtype. This finding matches the results from previous research which showed DLBCL as the most common NHL subtype which made up 40 to 50 percent of cases according to Roy et al. (2013) and Damle et al. (2017). The study by Pagaro et al. (2017) [16] found follicular lymphoma to be the most prevalent subtype in their study group thus showing how different regions exhibit distinct patterns of lymphoma subtypes. We found mixed cellularity to be the most common subtype of Hodgkin lymphoma (HL) in our research which matches the results from Khanday et al. (2019). The study by Arun Roy et al. (2013) found nodular sclerosis to be the most prevalent HL type, which indicates that environmental and genetic factors determine histological patterns.

The histological differences between the two groups received confirmation through immunohistochemical profiling. The majority of cases showed LCA positivity which confirmed their lymphoid origin, while B-cell NHL showed CD20 expression as its main feature according to Borgohain et al. (2020) [17] who showed that CD20 served both diagnostic and treatment purposes for B-cell cancers. The limited CD3 positivity results showed T-cell lymphomas existed in smaller numbers according to the findings of Hussain et al. (2019). Our research group found that classical Hodgkin lymphoma cases showed strong CD15 and CD30 expression which matched the immunophenotypic profile established by Mushtaque et al. (2019). Bcl-2 positivity in a subset of cases suggested anti-apoptotic mechanisms in certain lymphomas which matched previous research that showed Bcl-2 played a role in disease advancement and treatment resistance (Borgohain et al., 2020). The study found only minimal pancytokeratin expression which successfully ruled out the presence of epithelial cancers.

Our research findings from lymphoma subtypes showed links to specific immunohistochemical markers. Doctors use immunophenotyping to identify different lymphoma types which they determine through CD20 and LCA tests for NHL and CD15 and CD30 tests for HL. The results confirm the findings of Mushtaque et al. (2019) and Borgohain et al. (2020) who found similar relationships in their studied groups. The process of immunohistochemical profiling provides essential support both for correct diagnosis and for determining suitable therapies which are especially important during this time of monoclonal antibody and B-cell targeted treatment development.

The study shows that most lymphoma cases affect middle-aged men and NHL DLBCL serves as the most common subtype of the disease. The immunohistochemical profile showed that B-cell lineage represents the main lineage in NHL while CD15 and CD30 function as specific diagnostic markers for HL. The research shows similarities with earlier studies while also showing different patterns in age distribution and gender dominance and histological subtypes and immunophenotypic characteristics which demonstrate how lymphoma presents differently among various population groups. The research findings play a crucial role in improving diagnosis methods and enhancing patient treatment processes while they also contribute to future studies about lymphoma spread patterns and genetic research.

Conclusion

The present study concludes that lymphomas predominantly affect middle-aged males, with Non-Hodgkin Lymphoma (NHL) representing the majority of cases and Diffuse Large B-Cell Lymphoma (DLBCL) being the most frequent subtype. Histological evaluation, complemented by immunohistochemical (IHC) profiling, proved indispensable in distinguishing between NHL and Hodgkin Lymphoma (HL) and in accurately subclassifying lymphoma variants. IHC markers such as CD20 and LCA were strongly associated with B-cell NHL, while CD15 and CD30 were characteristic of HL, confirming their diagnostic utility. The integration of morphology with immunophenotypic data enhances diagnostic precision, informs targeted therapeutic strategies, and supports prognostic assessment. Overall, the study highlights the critical role of combined histological and IHC analysis in the effective diagnosis, classification, and clinical management of lymphomas.

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