



PATTERN OF LYMPH NODE LESIONS WITH SPECIAL REFERENCE TO IMMUNO-PHENOTYPING IN A RESOURCE-LIMITED SETTING

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ABSTRACT

Background: Lymphadenopathy is a frequent clinical finding with diverse etiologies ranging from reactive conditions to malignant neoplasms. Accurate diagnosis is essential, particularly in resource-limited settings where advanced diagnostic modalities may be selectively available. Histopathology remains the cornerstone of evaluation; however, immunophenotyping plays a critical role in precise classification of lymphoid lesions.

Objectives: To analyze the histopathological spectrum of lymph node lesions and to assess the diagnostic utility of immunophenotyping using immunohistochemistry and flow cytometry in a resource-limited setting.

Materials and Methods: Cases included underwent routine histopathological examination using hematoxylin and eosin staining. Immunohistochemistry was performed in selected cases using a targeted antibody panel. Flow cytometric immunophenotyping was applied in suspected lymphoid neoplasms where adequate fresh tissue was available.

Results: Non-neoplastic lesions constituted the majority of cases, with **reactive lymphadenitis** being the most common diagnosis, followed by **granulomatous lymphadenitis**. Lymphoid neoplasms accounted for a significant proportion, predominantly **B-cell non-Hodgkin lymphomas**, followed by T-cell lymphomas and classical Hodgkin lymphoma. Metastatic malignancies formed a smaller subset, with squamous cell carcinoma being the most frequent.

Conclusion: The study highlights the predominance of benign lymph node lesions in a resource-limited setting while emphasizing the critical role of immunophenotyping. Integration of histopathology with immunohistochemistry and flow cytometry significantly enhances diagnostic precision and supports optimal patient management.

Keywords: Lymphadenopathy; Lymph node biopsy; Immunohistochemistry; Flow cytometry; Lymphoma

INTRODUCTION

Lymphadenopathy is a common clinical presentation encountered across medical and surgical disciplines and represents a wide spectrum of pathological conditions ranging from benign reactive processes to malignant neoplasms. In developing and resource-limited settings, lymph node enlargement is frequently investigated because of its association with infections, immune-mediated

disorders, and malignancies that carry significant morbidity and mortality.¹ Accurate and timely diagnosis of lymph node lesions is therefore critical for appropriate patient management.

Histopathological examination of lymph node biopsies remains the gold standard for evaluating lymphadenopathy. Conventional microscopy allows classification of lesions into reactive, inflammatory, granulomatous, lymphoproliferative, and metastatic categories.² Neoplastic lesions, including lymphomas and metastatic carcinomas, though less frequent, require precise diagnosis due to their distinct therapeutic and prognostic implications.

However, reliance on morphology alone poses diagnostic challenges. Reactive lymphoid hyperplasia may mimic low-grade lymphomas, while certain lymphomas show subtle or overlapping histological features that cannot be reliably distinguished on hematoxylin and eosin staining alone.³ Misclassification or under-classification of lymphoid neoplasms can result in delayed treatment or inappropriate therapy. This limitation has led to increasing emphasis on ancillary diagnostic techniques to complement routine histopathology.

Immunophenotyping, primarily performed using immunohistochemistry (IHC) and, where available, flow cytometry, has become an indispensable tool in the evaluation of lymph node lesions. Immunophenotyping enables determination of cell lineage, assessment of clonality, and accurate subtyping of lymphoid neoplasms based on antigen expression profiles.⁴ Markers such as CD20, CD3, CD5, CD10, CD15, CD30, BCL2, BCL6, and Ki-67 play a pivotal role in distinguishing reactive from neoplastic processes and in classifying lymphomas according to the updated World Health Organization (WHO) classification of haematolymphoid tumours.⁵

The 5th edition of the WHO classification emphasizes an integrated diagnostic approach combining morphology, immunophenotype, genetic features, and clinical findings for accurate diagnosis of lymphoid neoplasms.⁶ Furthermore, immunophenotypic profiling provides prognostic information and guides targeted therapy, making it essential even in basic diagnostic settings.

MATERIALS AND METHODS

Study Setting & Design

A hospital-based descriptive observational study was conducted over a period of one year from January to December 2025 to evaluate the histopathological pattern of lymph node lesions with special reference to immunophenotyping. The study was carried out in the Department of Pathology of a tertiary care teaching hospital catering to a predominantly rural and semi-urban population. The study included patients of all age groups and both sexes who underwent lymph node biopsy and whose specimens were received in the Department of Pathology during the study period. All consecutive lymph node biopsy specimens received during the study period and fulfilling the inclusion criteria were included. A total of 120 cases were analysed based on convenient sampling technique used.

Inclusion Criteria

- All excised lymph node biopsy specimens received for histopathological examination
- Adequately preserved lymph node tissue
- Cases in which immunophenotyping by immunohistochemistry was performed where indicated

Exclusion Criteria

- Inadequate or autolysed specimens
- Recurrent or previously diagnosed cases of lymphoma already on treatment
- Lymph node aspirates without histological tissue

Specimen Processing

Lymph node biopsy specimens were fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin. Sections of 3–5 µm thickness were cut and stained with hematoxylin and eosin (H&E) for histopathological evaluation.

Histopathological Evaluation

All H&E-stained slides were examined under light microscopy. Lymph node lesions were classified based on architectural and cytological features into:

- Reactive lymphadenitis
- Granulomatous lymphadenitis
- Lymphoid neoplasms
- Metastatic malignancies

Histological diagnosis was rendered according to standard pathological criteria and correlated with available clinical details.

Immunophenotyping by Immunohistochemistry (IHC)

Immunohistochemistry was performed in selected cases where morphology alone was insufficient for definitive diagnosis or subclassification, particularly in suspected lymphomas. IHC was carried out on formalin-fixed paraffin-embedded sections using the polymer-based detection method. A limited but essential antibody panel was employed based on morphological suspicion and resource availability. Commonly used markers included:

- **CD3** (T-cell marker)
- **CD20** (B-cell marker)
- **CD5, CD10** (lymphoid differentiation markers)
- **CD15, CD30** (Hodgkin lymphoma markers)
- **Ki-67** (proliferation index)

Appropriate positive and negative controls were used. Immunoreactivity was interpreted based on staining intensity, pattern (membranous, cytoplasmic, or nuclear), and proportion of positive cells.

Diagnostic Criteria

Final diagnosis of lymphoid neoplasms was made using a combined assessment of histomorphology and immunophenotypic findings, following the World Health Organization (WHO) classification of haematolymphoid tumours (5th edition).

Data Collection & Statistical Analysis

Clinical details including age, sex, site of lymph node, and clinical diagnosis were obtained from requisition forms and medical records. Histopathological and immunophenotypic findings were recorded in a structured proforma. Data were entered into Microsoft Excel and analysed using SPSS version 21. Descriptive statistics were used to summarise findings. Categorical variables were expressed as frequencies and percentages.

RESULTS

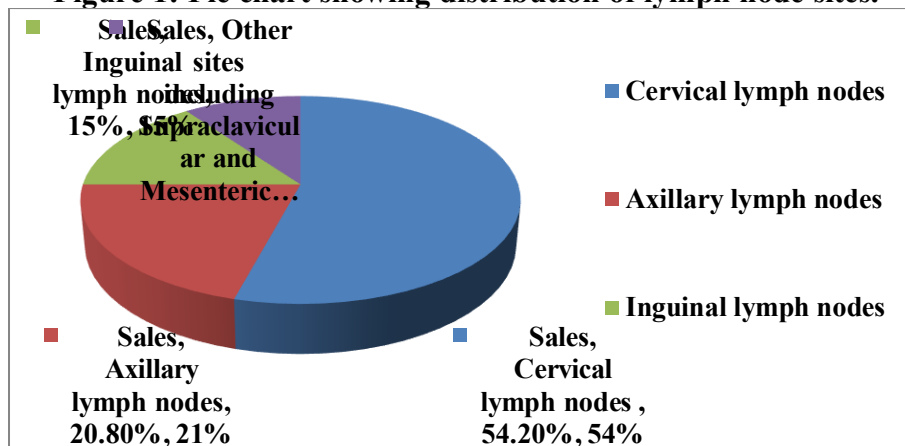
A total of 120 lymph node biopsy specimens were included in the study. All cases were subjected to routine histopathological examination. Immunophenotyping by immunohistochemistry (IHC) and/or flow cytometry was performed in selected cases where morphology alone was insufficient for definitive diagnosis.

Table 1: Age and Sex Distribution of Patients (n = 120)

Age Group (years)	Male	Female	Total (%)
≤20	10	8	18 (15.0)
21–40	24	18	42 (35.0)
41–60	26	14	40 (33.3)
>60	12	8	20 (16.7)
Total	72	48	120 (100)

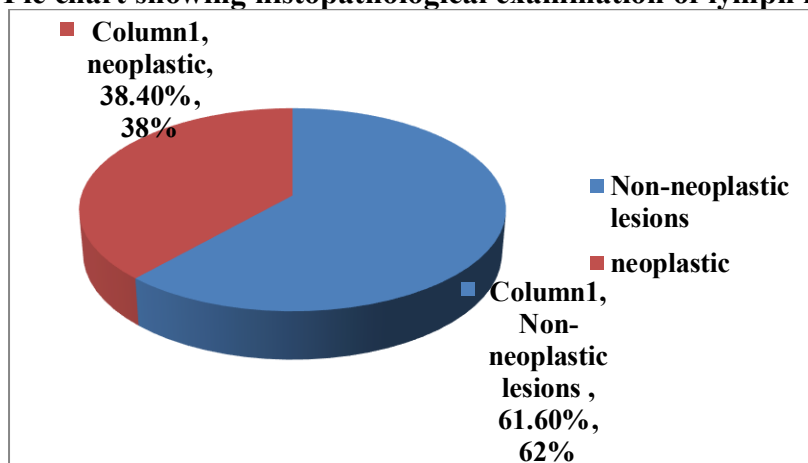
The age of patients ranged from 2 to 78 years, with a mean age of 42.6 ± 16.8 years. The majority of cases were seen in the third to fifth decades of life. There was a male predominance with 72 males (60%) and 48 females (40%), giving a male-to-female ratio of 1.5:1.

Figure 1: Pie chart showing distribution of lymph node sites.



The most common site of lymph node biopsy was cervical lymph nodes (54.2%), followed by axillary (20.8%), inguinal (15.0%), and other sites including supraclavicular and mesenteric nodes (10.0%).

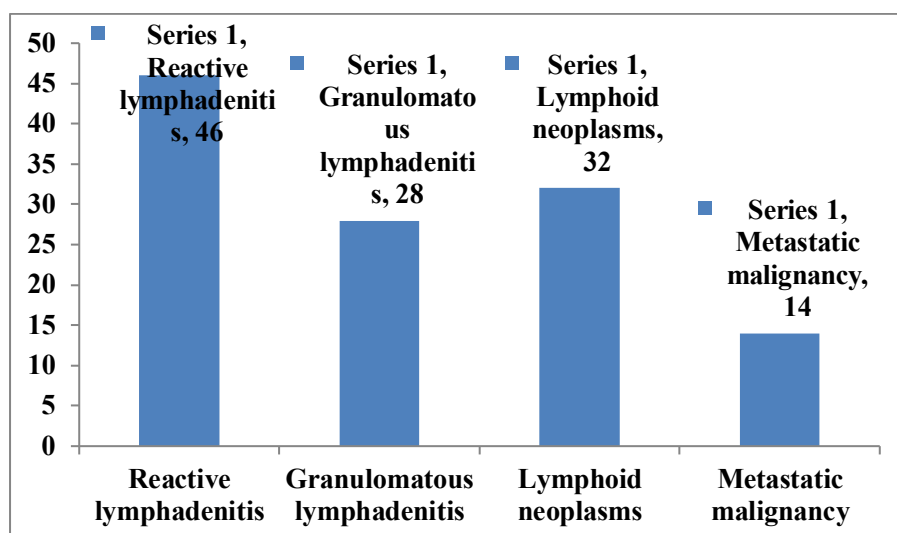
Figure 2: Pie chart showing histopathological examination of lymph node lesions



Based on histopathological examination, lymph node lesions were broadly classified into non-neoplastic and neoplastic categories.

Non-neoplastic lesions constituted 61.6% of cases, while 38.4% were neoplastic

Figure 3: Histopathological Diagnosis of Lymph Node Lesions (n = 120)



Majority of Lymph node lesions showed Reactive lymphadenitis followed by lymphoid neoplasms

Table 2: Immunophenotypic Classification of Lymphoid Neoplasms by IHC (n = 32)

Lymphoma Type	IHC Profile (Key Markers)	Number (%)
B-cell non-Hodgkin lymphoma	CD20+, CD3–	20 (62.5)
T-cell non-Hodgkin lymphoma	CD3+, CD20–	6 (18.8)
Classical Hodgkin lymphoma	CD15+, CD30+	6 (18.8)
Total		32

IHC was performed in 48 cases (40%), predominantly in suspected lymphoid neoplasms and diagnostically challenging reactive lesions.

Among lymphoid neoplasms (n = 32), IHC enabled lineage assignment and subclassification.

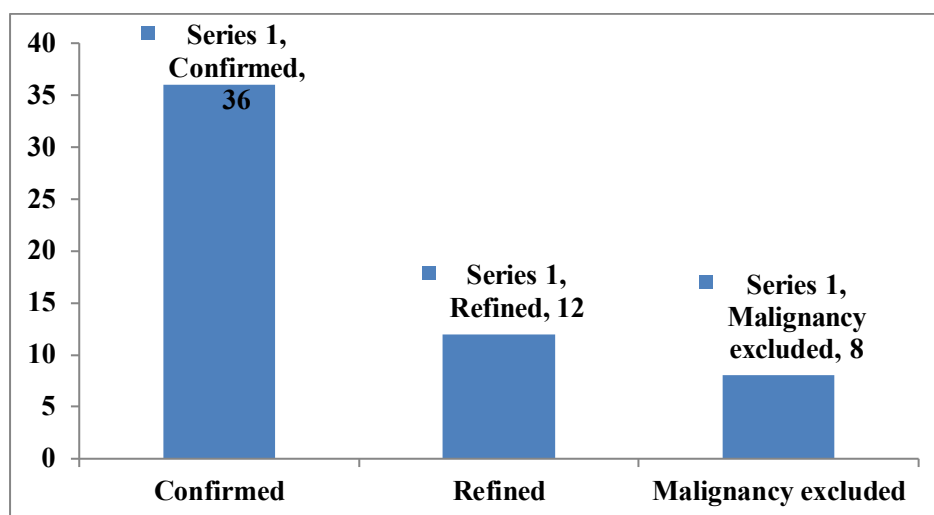
Ki-67 proliferation index was high (>40%) in 75% of high-grade lymphomas, aiding in grading and prognostication.

Table 3: Flow Cytometry Findings in Suspected Lymphoid Neoplasms (n = 22)

Parameter	Number of Cases (%)
B-cell clonality (κ/λ restriction)	14 (63.6)
T-cell immunophenotypic aberrancy	5 (22.7)
Polyclonal / reactive pattern	3 (13.7)
Total	22 (100)

Flow cytometry was performed in 22 cases (18.3%), where fresh tissue was available and lymphoma was suspected. Flow cytometry helped confirm clonality and lineage, especially in cases with equivocal morphology. Flow cytometry findings were concordant with histopathology and IHC in 90.9% of cases.

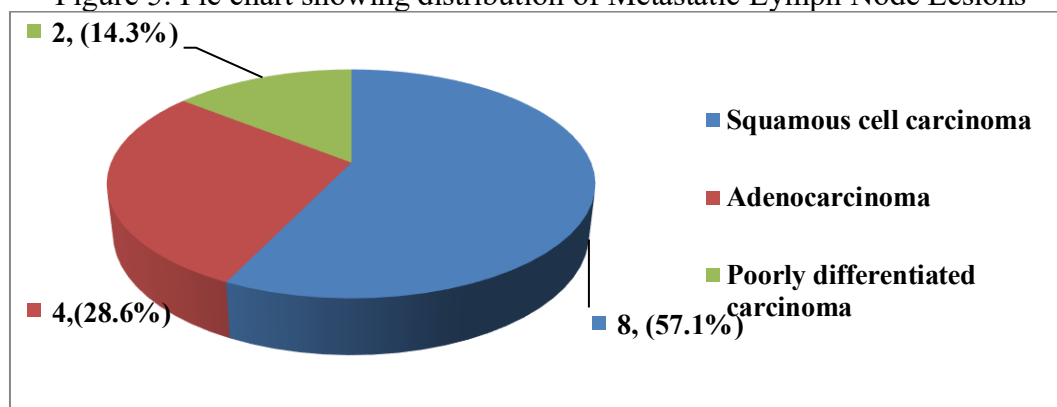
Figure 4: Bar diagram showing diagnostic contribution of immunophenotyping.



Immunophenotyping (IHC ± flow cytometry) contributed to:

- **Confirmation of diagnosis** in 36 cases (30%)
- **Change or refinement of diagnosis** in 12 cases (10%)
- **Exclusion of malignancy** in 8 morphologically suspicious cases

Figure 5: Pie chart showing distribution of Metastatic Lymph Node Lesions



Among the **14 metastatic cases**, the most common primary sites were:

- Squamous cell carcinoma – **8 cases (57.1%)**
- Adenocarcinoma – **4 cases (28.6%)**
- Poorly differentiated carcinoma – **2 cases (14.3%)**

IHC was useful in identifying the epithelial origin in poorly differentiated metastatic deposits.

DISCUSSION

Reactive lymphadenitis was the most common histopathological finding in our study, reflecting immune responses to localized or systemic infections. Similar observations have been reported by contemporary Indian and South Asian studies as quoted by Singh et al in 2023⁷ and Sharma et al in 2022⁸ which emphasize that reactive hyperplasia continues to account for a substantial proportion of lymph node biopsies in tertiary care hospitals. Granulomatous lymphadenitis, largely attributed to tuberculosis in endemic regions, was the second most common lesion. This pattern aligns with recent literature highlighting tuberculosis as a major cause of peripheral lymphadenopathy in resource-limited settings.⁹ The morphological overlap between granulomatous inflammation and certain lymphomas underscores the importance of careful histopathological evaluation and judicious use of ancillary techniques.

Lymphoid neoplasms comprised a significant proportion of cases in the present study, with B-cell non-Hodgkin lymphoma (NHL) being the most prevalent subtype, followed by T-cell lymphomas and classical Hodgkin lymphoma. This distribution is consistent with recent epidemiological data

indicating that B-cell lymphomas constitute approximately 70–85% of all lymphomas worldwide as quoted by Swerdlow et al in 2022.¹⁰ Immunohistochemistry (IHC) played a pivotal role in lineage determination and subtype classification, particularly in cases where architectural effacement or cytological atypia was subtle. The use of markers such as CD20, CD3, CD15, and CD30 enabled accurate classification in accordance with current WHO recommendations.¹¹

Flow cytometric immunophenotyping further enhanced diagnostic accuracy in selected cases of suspected lymphoid neoplasms. In our study, flow cytometry confirmed B-cell clonality and identified T-cell immunophenotypic aberrancies, supporting histopathological and IHC findings. Recent studies done by Craig et al in 2020¹² & Singh et al in 2022¹³ have demonstrated that flow cytometry significantly improves the detection of monoclonality and aberrant antigen expression, especially in small biopsies or cases with limited tissue availability. The concordance observed between morphology, IHC, and flow cytometry in the present study reinforces the complementary nature of these modalities.

Metastatic malignancies constituted a smaller but clinically important subset of lymph node lesions in our cohort. Squamous cell carcinoma was the most frequently encountered metastatic tumor, consistent with patterns reported in recent oncopathological studies.¹⁴ Histopathological examination, supported by IHC where required, facilitated accurate identification of metastatic deposits and exclusion of primary lymphoid malignancies.

The diagnostic contribution of immunophenotyping in the present study was substantial, with IHC and/or flow cytometry confirming, refining, or excluding malignancy in a significant number of cases. This finding echoes recent literature of Armitage et al in 2024¹⁵ emphasizing that reliance on morphology alone may lead to under- or misclassification of lymphoid lesions. In resource-limited settings, strategic application of a limited but targeted immunohistochemical panel, supplemented by flow cytometry when available, can optimize diagnostic yield while maintaining cost-effectiveness.

Recommendations

1. Routine incorporation of immunohistochemistry is recommended for accurate classification of lymph node lesions, especially lymphoid neoplasms.
2. Flow cytometric immunophenotyping should be utilized wherever feasible to improve detection of clonality and immunophenotypic aberrancies.
3. Integration of molecular diagnostic techniques, when resources permit, may further refine lymphoma classification and prognostication.

Limitations

1. The study was conducted at a single tertiary care center, which may limit the generalizability of the findings.
2. Although immunohistochemistry was performed in all relevant cases, flow cytometry could be applied only to selected specimens due to resource constraints.
3. A limited antibody panel was used in some cases, which may have restricted detailed subclassification of certain lymphoid neoplasms.

CONCLUSION

The present study demonstrates that non-neoplastic lesions, particularly reactive and granulomatous lymphadenitis, constitute the majority of lymph node pathologies in a resource-limited setting. However, a significant proportion of cases represent lymphoid and metastatic malignancies requiring accurate classification. Integration of histopathology with immunohistochemistry and flow cytometry substantially enhances diagnostic accuracy, enables precise immunophenotypic characterization, and supports appropriate clinical management of lymph node lesions.

Conflict of Interest: None

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