Original Article

Histopathological diagnosis of lymph node biopsies and subtyping of lymphomas based on immunohistochemistry at a tertiary care centre in South Kerala

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DOI
10.21276/apalm.3693

Article History
Received: 02-09-2025
Revised: 02-12-2025
Accepted: 17-12-2025
Published: 05-01-2026

How to cite this article
Markose A, Mathew S, et al. Histopathological diagnosis of lymph node biopsies and subtyping of lymphomas based on immunohistochemistry at a tertiary care centre in South Kerala. Ann Pathol Lab Med. 2026;13(1):A41-A48.

Abstract

Background: Lymph node enlargement is a clinical sign with uncertain definitive diagnosis since the causes are many. In view of the wide spectrum of the possible differential diagnoses, it is imperative for the clinicians and pathologist to have a knowledge of the common causes of lymphadenopathy in their locality.
Aim: To study the spectrum of histopathological diagnosis of lymph node biopsies received at a tertiary care centre in South Kerala and to find out the proportion of neoplastic and non-neoplastic lesions and to subtype lymphoma based on immunohistochemistry.
Results: The male to female ratio was 1.1:1 with a slight male preponderance. The most common site biopsied was the cervical lymph node. Non-neoplastic lesions were more common and comprised 71% of all biopsies. Overall, the most common lesion was reactive lymphadenopathy. Tuberculous lymphadenitis was the next common lesion with highest incidence in individuals aged 30-39 years showing a female preponderance. Among neoplastic lesions, metastasis to lymph node showed a slight predominance over lymphoproliferative disorders. Metastasis from adenocarcinoma was the most common. All metastatic lesions were in the age group more than 30 years with a male preponderance. Non-Hodgkin's lymphoma was more common than Hodgkin's Lymphoma. Follicular lymphoma was the commonest among NHL. Among the 17 cases of NHL, 15 of the cases could be subtyped with the available basic IHC panel, rest 2 cases could not be precisely subtyped. All 3 cases of Hodgkin's Lymphoma were males with Classic Hodgkin's Disease- Mixed Cellularity type.
Conclusion: In the present study of 138 lymph node biopsies, non-neoplastic lesions were more frequent than neoplastic lesions, with reactive lymphadenitis being the most common diagnosis, followed by tuberculous lymphadenitis. Among neoplastic conditions, metastatic deposits—predominantly from adenocarcinoma—were observed more frequently than lymphomas, with a predilection for older males. Non-Hodgkin's lymphoma was more frequent than Hodgkin's lymphoma, with follicular lymphoma being the commonest subtype identified. These findings underscore the predominance of non-neoplastic causes of lymphadenopathy and reaffirm the critical role of histopathology, supplemented by immunohistochemistry, in arriving at a definitive diagnosis—particularly in cases where lymphoproliferative disorders are suspected.

Keywords: lymph node; reactive change; tuberculosis; Hodgkin's lymphoma; Non Hodgkin's Lymphoma; Metastasis

Introduction

Lymphadenopathy is a frequently encountered clinical condition with a wide range of underlying etiologies, including reactive, infectious, and neoplastic processes[1, 2]. Histopathological examination remains the gold standard for definitive diagnosis, especially when clinical and radiological assessments are inconclusive [3, 4]. In regions with a high prevalence of tuberculosis and limited healthcare access, infections such as tuberculous lymphadenitis continue to be a leading cause of peripheral lymph node enlargement [5, 6]. At the same time, lymphomas and metastatic malignancies are significant neoplastic contributors [7, 8, 9]. This study underscores the diagnostic value of lymph node biopsies in distinguishing between reactive, infectious, and malignant pathologies, thereby aiding in timely and appropriate clinical management[2, 10].

Materials and Methods

This is a prospective study conducted at a Government Medical College in South Kerala for a period of 18 months. The permission from the institutional review board was obtained for this study on 15/12/2016, IRB approval number: EC61/2016. The method of data collection did not pose any risk or harm to the cases as there was no interference with treatment. The patient identity and confidentiality were well maintained. As it was a prospective study with review of reports, there were no ethical issues and it was approved by an expedited review of the Institutional Ethics Committee. No funding was obtained for the study. This study was conducted according to the ethical guidelines by the Declaration of Helsinki and other guidelines like Good clinical Practice Guidelines and those established by ICMR.

Participants

Inclusion criteria: All lymph node excision biopsies specimen received at a government medical college in South Kerala during the study period (January 2017- June 2018). Exclusion criteria: Lymph node dissection done as a part of a staging procedure. There was a total of 138 lymph node excision biopsies received during the study period of 18 months (January 2017- June 2018) and all the cases were included in the study.

Aims and Objectives

To study the spectrum of histopathological diagnosis of lymph node excision biopsies received at a Government medical college in South Kerala during the study period and to subtype lymphomas based on immunohistochemistry.

Study Methodology: Descriptive prospective study.

Study Procedure

Clinicodemographic data was obtained by reviewing the patient records. Formalin fixed tissue was grossed and representative sections were taken in case of large lymph nodes. In smaller lymph nodes up to two cm size the entire tissue was processed in the automated tissue processor. Tissues were embedded in paraffin wax, and 4 micrometer sections were cut. Special stains like Acid Fast Bacilli (AFB) stain, Periodic Acid Schiff (PAS) stain, Gomori Methamine Silver (GMS) stain, Giemsa, Reticulin stains were done in specific cases. Non-neoplastic lesions of lymph nodes were diagnosed based on characteristic histomorphological criteria. Reactive lymphadenitis was identified when the lymph node showed follicular hyperplasia, paracortical hyperplasia or sinus histiocytosis. Tuberculous lymphadenitis was diagnosed in the presence of caseating granulomatous inflammation. Kikuchi–Fujimoto disease was recognized by necrotizing granulomatous inflammation with an absence of neutrophils. Dermatopathic lymphadenitis was diagnosed when there was marked paracortical expansion accompanied by melanin-laden macrophages. Toxoplasma lymphadenitis was identified by the presence of follicular hyperplasia along with sinusoidal monocytoid B-cell proliferation and microgranulomas. BCG lymphadenitis was considered in cases with a history of recent BCG vaccination showing granulomatous inflammation on histology. Dirofilariasis was diagnosed when lymph nodes demonstrated cross-sections of adult *Dirofilaria* worms within the nodal tissue. IHC panels for lymphoma were decided based on the cell morphology described in Tables 1-4. All lymphomas were classified according to the standard World Health Organization classification of Hematolymphpoid malignancies. IHC markers used – CD45, CD20, CD3, CD15, CD30, CD5, CD23, ALK, Cyclin D1, CD10, Bcl2, CD138, Ki67, CK using DAKO LSAB-2 ® system HRP. All Immunohistochemistry markers were done using internal controls.

Outcome

We aimed to reveal the relative frequencies of neoplastic and non-neoplastic lymph node lesions, identify the most common histopathological patterns in different age groups and gender, and highlight the diagnostic utility of lymph node biopsy in establishing definite diagnosis for lymphadenopathy.

Statistical Analysis

Descriptive statistics was used to express proportions. Continuous variables were expressed as mean (SD) or Median (IQR) based on normality. As it was a descriptive study which included all cases in the study period, a calculation of sample size was not applicable. All statistical analysis were done using Microsoft Excel and SPSS software 22.

Results

The most common site to be biopsied was the cervical lymph node and there was a male preponderance. Non neoplastic lesions constituted 71% while neoplastic lesions constituted 29% of the cases. The most common lesion overall was reactive change lymph node which was followed by tuberculous lymphadenitis.

Among neoplastic lesions, metastasis to lymph node showed a slight predominance over lymphoproliferative disorders. Metastasis from adenocarcinoma was the most common metastatic lesion. All metastatic lesions were in the age group more than 30 years with a male preponderance. Non Hodgkin's lymphoma was commoner than Hodgkin's Lymphoma with follicular lymphoma being the most common type.

Among the 17 cases of NHL, 15 of the cases could be subtyped with the available basic IHC panel in our department, rest 2 cases could not be precisely subtyped. All 3 cases of Hodgkin's Lymphoma were of Classic Hodgkin's Disease- Mixed Cellularity type and all were males.

Discussion

During the study period 138 lymph node biopsies were analysed. Out of these 73 were males (52.9%) and 65 were females (47.1%) showing a male to female ratio of 1.1:1, males and females being equally affected with a slight male preponderance similar to the study by Roy et al.[7], while in most other studies there was a slight female preponderance.[3, 5, 7]. This difference could be attributed to the fact that the location of this study was in rural population where female patients less often visit tertiary health care compared to an urban population.

Patient's age ranged was from 10 months to 82 years in case of males and was from 4years to 71 years in case of females. Maximum number of cases were seen in the age group of 10- 19 years (30 cases, 21.73%) and the least number were seen in the age group below 10 years (6 cases, 4.3%). The finding was consistent with the study by Roy et al.[7] and Albasri et al.[8]

The most common site biopsied was the cervical lymph node followed by axillary lymph node representing (62.31%) and (15.2%) respectively. This was in concordance with all studies conducted in the past, in both adults and children. This is because these lymph nodes drain the most commonly affected and inflamed region of the body as well as due to easy accessibility of the cervical lymph node for biopsy. [4, 7, 11, 12, 13] Mediastinal lymph node biopsies were conspicuously absent, which may be due to the difficulty in accessing the mediastinal nodes. In all cases of lung lesions associated with hilar lymphadenopathy, the primary lung lesion was more frequently biopsied, owing to its greater accessibility via bronchoscopy compared with the hilar lymph nodes. The least common sites were intra-abdominal (Ileal), Epitochlear and Infraclavicular regions -1 biopsy each was received from these sites and all 3 were lymphoproliferative disorders namely Non-Hodgkin's Lymphoma.

In the present study non neoplastic lesions (98 cases, 71.01%) were more common compared to neoplastic lesions (40 cases, 28.98%) which is similar to study by Kamat GC[3] with 88.92% were non neoplastic and 11.08% were neoplastic. (Figure 1) Similar results were also seen in study by Halder et al.[14] and AL-Ali et al.[15]; but malignant lesions were more common in the study done by Roy et al.[7], Albasri et al.[8] and Özkan et al.[16] The ratio of non-neoplastic to neoplastic lymph node lesions varies greatly with the kind of medical setting for example, in family practice or general medical practice, malignancies represent only about 1.1% of lymph node lesions, whereas at referral centers their frequency is 40% to 60%.[2, 17]

Among the non-neoplastic lesions, reactive hyperplasia was by far most common accounting for 36.95% (51 cases).(Figure 3) The lesion was found in all age groups with age group10-19 years being most frequent. Reactive hyperplasia was closely followed by tuberculous lymphadenitis. (Figure 2)

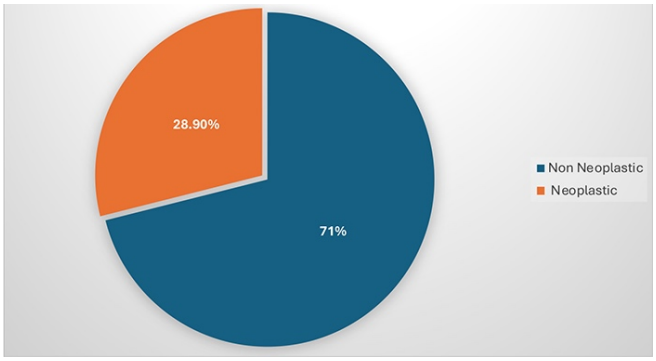

Figure 1: Proportion of neoplastic and non-neoplastic lesions.

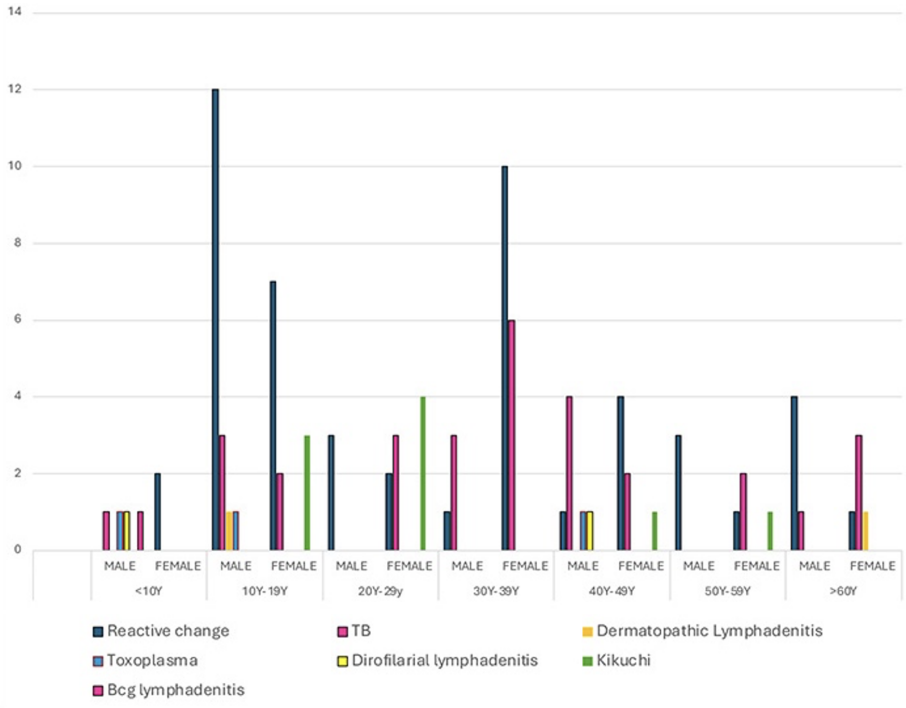

Figure 2: Age and gender wise distribution of non neoplastic lesions.

Tuberculous lymphadenitis was the second most common histologic pattern observed among the non-neoplastic lesions (30/98, 21.7%). Females were more commonly affected (18/30, 60%). 76.67% (23/30) of patients were younger than 50 years, with a peak incidence in the age group between 30 to 39 years. (Figure 3)

The knowledge of the pattern of lymphadenopathy in a given geographical region is essential for making a confident diagnosis or suspecting a disease. Tuberculosis is one of the most common cause of lymphadenopathy in developing countries such as Nepal, Bangladesh, India and should be considered in every case of granulomatous lymphadenopathy unless proved otherwise. It has been reported by several authors that tuberculosis is one of the predominant cause of lymph node enlargement in adults in tropics like India. Despite improvement in the living standard in general, significant reduction in the incidence of tuberculosis in Asian population has not occurred correspondingly. However, in western countries tuberculosis ranks third as a cause of cervical lymphadenopathy, this might indicate difference in living standards and socioeconomic conditions. A study by Pagaro PM et al.[5] and Gorle VK et al[6] found tuberculosis in 20.5% and 51%, respectively.

Other granulomatous lesions included two cases of dirofilarial lymphadenitis (Figure 5), three cases of toxoplasmosis and one case of BCG lymphadenitis.

Other non-neoplastic causes of lymph node enlargement were Kikuchi Fujimoto disease (9 cases, 6.52%) (Figure 5), Dermatopathic Lymphadenitis (2 cases, 1.45%).

Among neoplastic lesions (40 cases, 28.98%), more than half (21/40, 52.5%)were metastases to lymph node and the rest were lymphoproliferative disorders. Non-Hodgkins lymphoma was more common (16/19, 84.21%) than Hodgkins lymphoma (3/19, 15.78%). (Figure 4). Studies conducted by Mohan et al[18] , Dalme et al[4], reported 13.34%, 16.31% cases of

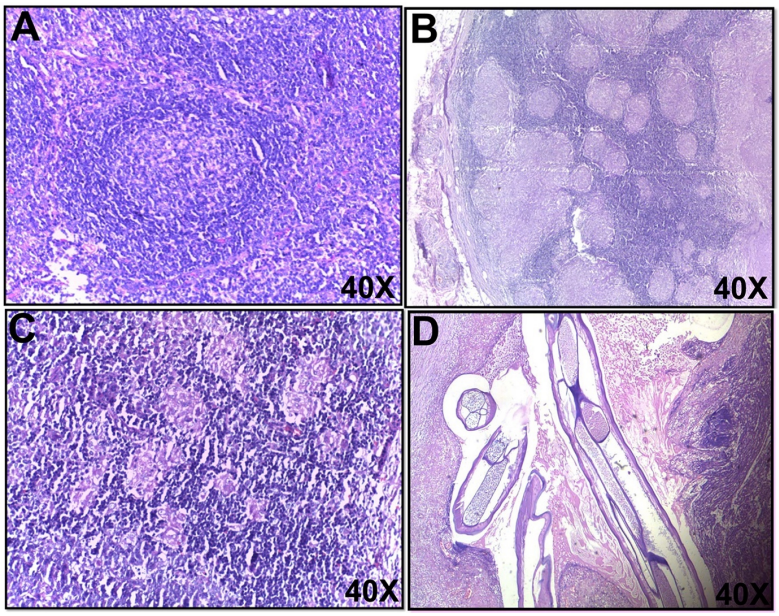

Figure 3: Histomorphology of non neoplastic lymph node lesions: A Reactive Lymphoid hyperplasia, B Tuberculous lymphadenitis, C Toxoplasma Lymphadenitis, D Dirofilarial lymphadenitis.

metastasis to lymph node which was corresponding with our study. There was male predominance with a male: female ratio of 1.3:1 which was comparable. Metastatic deposits was commonly observed in patients above 40 years of age in our study which is similar to Shrestha AK et al[19]. Most common was metastasis from adenocarcinoma as observed in the study by Roy et al[7] and Albasri et al[8].(Figure 5) Malignancies have been the predominant cause of lymphadenitis in developed countries than developing countries like India because of racial and genetic factors.

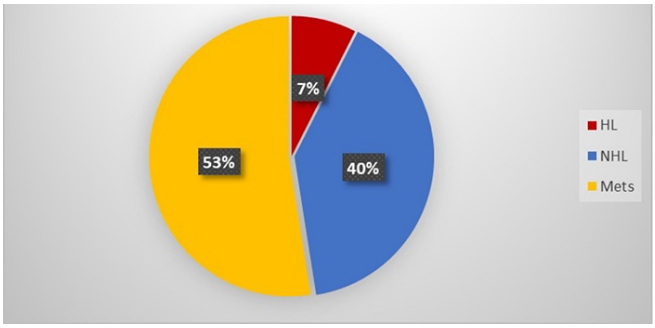

Figure 4: Distribution of neoplastic lesions.

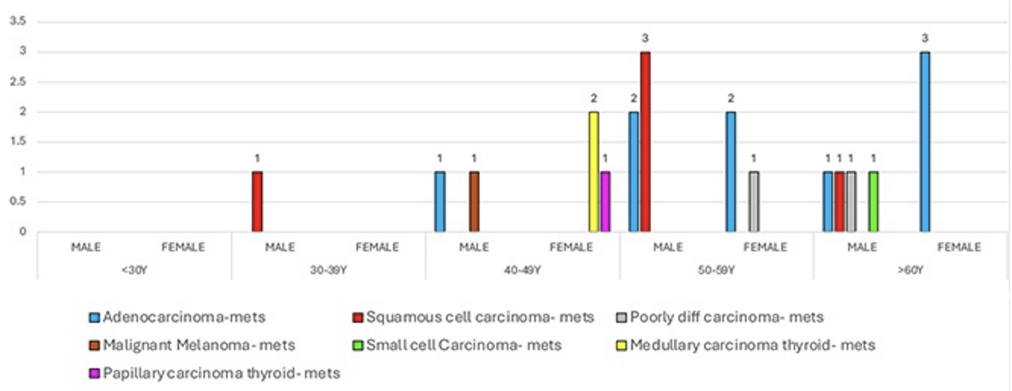

Figure 5: Age and gender wise distribution of metastatic lesions.

In our study, lymphoproliferative disorders constituted 13.76% of the cases. In a study conducted by Panchal Jaimin and Pushpalatha Pai [20] reported 15.6% of reported cases were lymphoma and was comparable with our study. But in contrast in studies by Roy A et al [7], Sinclaire S et al[21] and Mohan A et al[18] lymphoproliferative disorders constituted 44.5%, 63.29% and 25.9% respectively.

Among the lymphoproliferative disorders, Non-Hodgkin's lymphoma was more common than Hodgkin's Lymphoma (Figure 6). NHL was found to be the commonest type of lymphoma in all of the similar Indian and International studies[7, 8, 5, 4]. Also in the western world NHL was found to be more common than Hodgkin's Lymphoma.[22] The higher proportion of NHL in the Western world than here in India, may be partly explained by racial and genetic factors as comparative studies in the United States document higher incidence among Whites than Blacks.

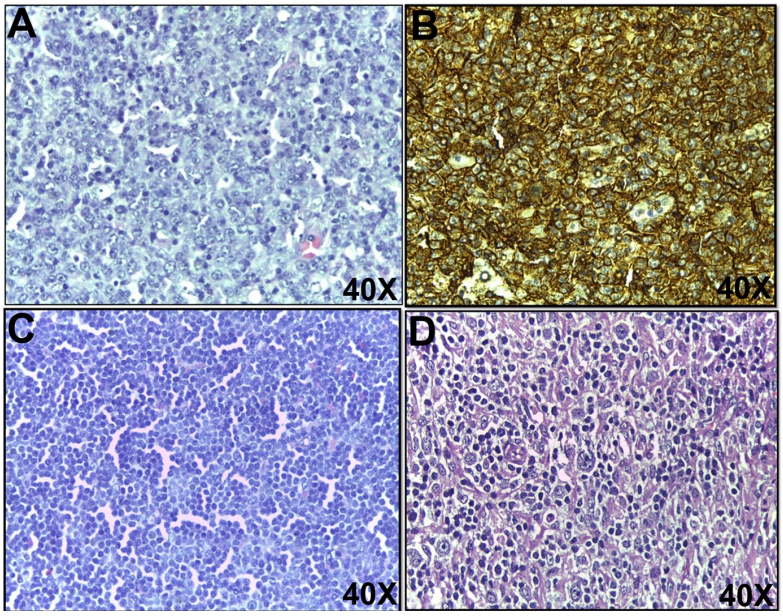

Figure 6: Histomorphology and immunohistochemistry of lymphomas: A-Diffuse large B-cell lymphoma, B-Immunohistochemistry CD-20 diffuse positivity, C-Small lymphocytic lymphoma, D-Hodgkin lymphoma-Mixed cellularity.

Among the Non-Hodgkin's Lymphoma, B cell type (14 cases, 10.1%) was more common than T cell type (2 cases, 1.44%) comparable to most other studies[5]. The most common type of NHL was the follicular lymphoma in this study similar to the studies by Pagaro et al[5]. However in some studies like the study by Roy et al[7], Albasri et al[8] , AL-ALI Zainab A [15] ,there was a higher incidence of aggressive NHL especially DLBCL. It may be related to the aetiology of DLBCL such as immune deficient conditions and their treatments which in most instances caused aggressive NHL, and we should consider that a comparative excess of DLBCL resulted in a deficit of follicular lymphoma. In our study, we found 4 cases of DLBCL, out of which one was an anaplastic variant of DLBCL.(Table 3) There was one case of B cell lymphoma which could not be typed into specific subtype with the help of the morphology and basic markers, hence was reported as B cell lymphoma, low grade.(Table 2)

There were just 2 cases of T cell lymphoma, 1 was a case of Anaplastic T cell Lymphoma and the other cases was reported as T cell lymphoma for which further typing was not possible due to the non-availability of advanced IHC markers.

There were no cases of precursor neoplasms(T or B lymphoblastic lymphomas) which may be due to the smaller sample size and also due to the fact that most cases of suspected lymphomas especially in paediatric age groups are referred to Oncology Institutes for further evaluation and treatment.

There were 3 cases of Hodgkin's Lymphoma, all were males and was of Mixed Cellularity subtype similar to the studies by Gupta et al,[23] Rahman et al[24] Albasri et al[8]. Whereas few other studies reported Nodular Sclerosis as the most common subtype.[7, 15]. However, the rate varies greatly among other geographical regions and NSCHL is more common in resource-rich than in resource-poor areas, and the risk is highest among those with high socioeconomic status. MCCHL is more frequent in patients with HIV infection and in developing countries.[25](Table 1).

Due to the non-availability of few IHC markers like Bcl6 and MUM1, further subtyping of DLBCL into Germinal centre B cell subtype and Activated B cell subtype could not be done.

Conclusion

Lymphadenopathy constitutes a common and clinically significant presentation of various diseases, affecting individuals across all age groups and sexes. In this study, non-neoplastic lesions (71%) markedly outnumbered neoplastic lesions (29%), with reactive lymphadenitis representing the single most frequent diagnosis, followed by tuberculous lymphadenitis, particularly in the 30–39-year age group where a female preponderance was noted. Within the neoplastic spectrum, metastatic lesions slightly exceeded lymphoproliferative disorders in frequency, with metastatic adenocarcinoma as the dominant

Table 1: Subtyping of lymphomas based on immunohistochemistry: Lymphoma with polymorphous population.

Age & Gender	Lymphoma	CD15	CD30	CD20	CD45	ALK-1
34/M	CHD-MC	+	+	-	-	-
18/M	ALCL	-	+	-	+	+
46/M	CHD-MC	+	+	-	-	-
48/M	NHL- T cell type	-	-	-	-	-
71/M	CHD-MC	+	+	-	-	-

CHD-MC- Classic Hodgkin Disease- Mixed cellularity, ALCL- Anaplastic Large cell Lymphoma, NHL- Non Hodgkin Lymphoma

Table 2: Lymphomas with monomorphic small cells.

Age/ Gender	Lymphoma	CD20	CD5	CD23	CyclinD1	Bcl2	CD10	Ki 67
62/M	SLL	+	+	+	-	-	-	-
42/M	FL grade II	+	-	-	-	+ (nodules)	+	-
47/M	FL grade I	+	-	-	-	+ (nodules)	+	-
49/M	FL grade I	+	-	-	-	+ (nodules)	+	-
80/M	SLL	+	+	+	-	-	-	-
61/M	FL grade I	+	-	-	-	+ (nodules)	+	-
47/M	FL grade II with diffuse areas	+	-	-	-	+ (nodules)	-	-
60/M	MCL transformation to DLBCL	+	+	-	+	-	-	-
70/ M	B cell lymphoma-Low grade	+	-	-	-	+ (diffuse)	-	<5%

FL- Follicular Lymphoma, MCL- Mantle cell lymphoma

Table 3: Lymphomas with monomorphic large cells with diffuse pattern.

Age/Gender	Lymphoma	CD20	CD138	Cyclin D1	CD30
60/M	DLBCL	+ (diffuse)	-	-	-
71/F	DLBCL	+ (diffuse)	-	-	-
66/F	Anaplastic variant of DLBCL	+ (diffuse)	-	-	+
40/M	DLBCL	+ (difuse)	-	-	-

DLBCL – Diffuse Large B cell lymphoma

Table 4: Lymphomas with blastic morphology.

Lymphoma	CD20	CD5	CyclinD1	CD23
MCL blastoid variant	+	+	+	–

MCL - Mantle cell lymphoma

metastatic pathology. All metastatic lesions occurred in patients above 30 years of age, with a clear cut male predominance. Non-Hodgkin's lymphoma surpassed Hodgkin's lymphoma, with follicular lymphoma emerging as the most frequent NHL subtype. All HL cases were classical Hodgkin's lymphoma, mixed cellularity type, observed exclusively in males. These observations underscore the pivotal role of histopathological examination of lymph node biopsy as the definitive diagnostic modality in evaluating lymphadenopathy. Given the broad etiological spectrum and the prognostic implications of timely diagnosis, early biopsy coupled with precise pathological categorization is indispensable for directing optimal patient management and improving clinical outcomes.

Acknowledgements: Nil

Funding: No funding was obtained for the study.

Competing Interests: No conflict of interest.

References

1. Kumar V, Abbas AK, Aster JC. Robbins and Cotran Pathologic Basis of Disease. 9th ed. Philadelphia, PA: Elsevier/Saunders; 2015.
2. Ferrer R. Lymphadenopathy: Differential diagnosis and evaluation. Am Fam Physician. 1998;58(6):1313–20.
3. Kamat GC. Histopathological pattern of lymphadenopathy in rural India. Int J Pathol. 2006;5(2):1–4.
4. Dalme JB, et al. Histopathological study of lymphadenopathy – a tertiary care hospital experience. J Evid Based Med Healthc. 2017;4(92):5623–28.
5. Pagaro PM, et al. Histopathological spectrum of lymphadenopathy with special reference to tuberculosis. Int J Health Sci Res. 2014;4(7):36–40.
6. Gorle VK, et al. Evaluation of lymphadenopathy by histopathological examination. Int J Res Med Sci. 2016;4(11):4754–4758.
7. Roy A, et al. Clinicopathological correlation of lymphadenopathy. Indian J Pathol Oncol. 2016;3(1):55–58.
8. Albasri A, et al. Histopathological pattern of lymph node biopsies in Western Saudi Arabia. Saudi Med J. 2014;35(11):1368–73.
9. Rahman K, et al. Histopathological pattern of Hodgkin's lymphoma in a Pakistani population. Pak J Med Sci. 2008;24(2):276–280.
10. Sinclaire S. Lymphadenopathy: Pathologic perspective. Arch Pathol Lab Med. 2002;126:105–109.
11. Apoorva Saraswat, Akash Rajender, Kunal Purohit, R. M. Jaiswal, Ritu Sharma, Divya Dubey. Lymph Node Biopsy: Spectrum and Clinical Significance as Diagnostic tool at Tertiary Care Centre. Journal of Evolution of Medical and Dental Sciences 2015; 4(6):1008-1014.
12. Vachhani AB, Bhuva kaushik, Jasani JH, Tandon RK. Histopathological study of lymph node biopsy. Int J of Biomed & Adv Res 2013;4(11):790–5.
13. Olu-Eddo AN, Ohanaka CE. Peripheral lymphadenopathy in Nigerian adults. Journal of Pakistan Medical Association 2006; 56(9):405-408.
14. Halder S, Saha B, Sarkar D, Ghosh S. Etiological study of generalized lymphadenopathy in a tertiary care hospital. International Journal of Research in Medical Sciences 2016;3542–8.
15. AL-Ali ZA. Patterns of lymph node pathology using biopsy evaluation tool for lymphadenopathy: a retrospective descriptive study conducted at Al Hussein Medical City 2014–2016. Iraq Medical Journal. 2017; 1(4):102-105.
16. Özkan EA, Göret CC, Özdemir ZT, Yanık S, Göret NE, Doğan M, et al. Evaluation of peripheral lymphadenopathy with excisional biopsy: six-year experience. Int J Clin Exp Pathol 2015;8(11):15234–9.
17. Bazemore AW, Smucker DR. Lymphadenopathy and malignancy. Am Fam Physician 2002;66(11):2103–10.
18. Mohan A, Reddy MK, Phaneendra BV, Chandra A. Aetiology of peripheral lymphadenopathy in adults: analysis of 1724 cases seen at a tertiary care teaching hospital in southern India. Natl Med J India 2007;20(2):78–80.
19. Shrestha ML, Shrestha AK, Chalise PR. Lymph Node Biopsies: A Hospital Based Retrospective Study. Journal of Nepal Medical Association 2009; 48(176): 306-9.
20. Jaimin P, Pai P. Spectrum of pathologic lesions in superficial lymph node biopsies –A one and half year study. International Journal of Biomedical And Advance Research 2014; 05 (09): 435-438.
21. Sinclair S, Beckman E, Ellman L. Biopsy of enlarged, superficial lymph nodes. JAMA 1974;228(5):602–3.
22. Hartge P, Devesa SS, Fraumeni JF. Hodgkin's and non-Hodgkin's lymphomas. Cancer Surv 1994;19–20:423–53.
23. Gupta D, Thaker BD, Suri J. Pattern of lymphadenopathies in a tertiary care hospital in Jammu, India. Global Journal for Research Analysis 2017; 6(7): 9-11.
24. Rahman MA, Biswas MMA, Siddika ST, Sikder AM. Histopathological Evaluation of Lymph Node Biopsies: A Hospital Based Study. Journal of Enam Medical College 2012; 2(1): 8-14.
25. Alaggio R, Amador C, Anagnostopoulos I, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. WHO Classification of Tumours. Lyon (France): International Agency for Research on Cancer; 2022.