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Original Research Article

UTILITY OF IMMUNOHISTOCHEMISTRY IN THE DIAGNOSTIC WORKUP OF LYMPHOMAS

Vandana Bhatti[1], Nalini Calton[2], Kanwardeep Singh Kwatra[3]

[1]Associate Professor, Department of Pathology, Christian Medical College and Hospital, Ludhiana, India.
[2]Professor, Department of Pathology, Christian Medical College and hospital, Ludhiana, India.
[3]Senior Consultant, Department of Pathology, Onquest laboratories limited, Mohandai Oswal Hospital, Ludhiana, India.

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Corresponding Author:
Dr. Vandana Bhatti,
Email: vandanarajeshwar@gmail.com.

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Abstract

Background: Lymphomas are malignancies of the lymphatic system, broadly subdivided into Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) which are then further sub-classified. Immunohistochemistry (IHC) plays a three-pronged role in the diagnosis and management of lymphomas namely, histological sub-typing, prognostication, and guiding targeted therapy. **Aim:** The study aims to study the spectrum of lymphomas and to analyze the role of IHC in their accurate diagnosis and sub-classification. **Materials and Methods:** This 3-year cross-sectional study included all cases diagnosed as lymphoma on tissue biopsies. IHC markers were selected from a panel, based on morphologic assessment and differential diagnosis. IHC helped in the confirmation of diagnosis and further sub-classification. **Results:** There were 62 cases of lymphoma out of which 56 (90.3%) were NHL and 6 (9.7%) were HL. IHC was performed on all the cases. Among NHL, 87.5% were of B-cell (n=49/56) and 12.5% (n=7/56) of T-cell lineage. DLBCL comprised the maximum cases accounting for 52.7% (29/56) followed by anaplastic large cell lymphoma accounting for 10.9% (6/56). The male to female ratio among NHL was 1.8:1 with a mean age of 55. All cases of HL were classical Hodgkin lymphoma with a male to female ratio of 2:1 and a mean age of 35 years. **Conclusion:** Immunohistochemistry has emerged as a valuable adjunct to hematoxylin and eosin (H&E) morphology for accurate diagnosis and sub-classification of lymphomas. A minimum set of IHC markers namely, CD45, CD3, CD4, CD8, CD5, CD7, CD10, CD20, CD21, CD23, PAX-5, CD79a, CD15, CD30, BCL2, BCL6, OCT2, MUM1, cyclin D1, c-MYC, Ki67, Alk-1, EBV(LMP-1), CD34, TdT, CD99, CD138, kappa, and lambda light chain is an essential requirement for any standard histopathology laboratory.

INTRODUCTION

Although in general, a well-stained Hematoxylin and Eosin section remains the gold standard for histopathological diagnosis, immunohistochemistry (IHC) is now playing an increasing role in more accurate tissue diagnosis. It is a relatively simple and cost-effective technique.[1]

Immunohistochemistry is a method for localizing specific antigens in tissues based on antigen-antibody reactions. The reaction is identified by using a secondary labeling method.[2] The use of IHC in diagnostic pathology has expanded so much that one or more IHC stains have become essential and routinely used in surgical pathology, especially with respect to tumor diagnosis, classification, and prognostication.[3] IHC plays a three-pronged role in diagnosing and managing lymphomas: histological sub-typing, prognostication, and identifying cases for targeted therapy.[4] IHC allows the objective identification of specific phenotypic characteristics associated with different lymphoid proliferations.[5] Previous classification systems based purely on morphology are obsolete. The present study highlights the spectrum of lymphomas diagnosed at a tertiary care hospital in North India and analyzes the role of IHC in their accurate diagnosis and sub-classification.

MATERIALS AND METHODS

This 3-year cross-sectional study included all cases diagnosed as lymphoma on tissue biopsies. IHC markers were selected based on the morphologic assessment which took into account the architectural alteration in the lymphoid compartments and cell morphology. Lymphoma IHC panel included CD45, CD3, CD4, CD8, CD5, CD7, CD10, CD20, CD21,

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CD23, PAX-5, CD79a, CD15, CD30, BCL2, BCL6, OCT2, MUM1, Cyclin D1, c-MYC, Ki67, Alk-1, EBV(LMP-1), CD34, TdT, CD99, CD138, kappa and lambda light chain. For cases suspected to be NHL, an initial panel was applied to broadly categorize the lymphoma as B/T cell lineage and then other markers were selected from the above panel depending on the lineage identified. For cases suspected to be HL, a panel including LCA, CD3, CD20, PAX-5, CD30, CD15 and EBV (LMP-1) was used. IHC was performed using the Novolink HRP-linked Polymer Detection System with DAB chromogen from Leica Biosystems. Antigen retrieval was done by high temperature and pressure method. Descriptive analysis was performed and data were analyzed using percentages and proportions. The study was approved by the institutional research and ethics committee.

RESULTS

There were 62 cases of lymphoma out of which, 56 (90.3%) cases were diagnosed as non-Hodgkin lymphoma and 6 (9.7%) as Hodgkin lymphoma. Among NHL, the male-to-female ratio was 1.8:1(M=36, F=20) and age ranged from 4 to 90 years with a mean age of 55. The youngest patient among NHL was diagnosed with Burkitt lymphoma and the eldest was diffuse large B-cell lymphoma. There were 67.8% (38/56) nodal and 32.1% (18/56) extranodal lymphomas. The distribution of lymphomas according to the site of involvement is given in Table/fig1. The most common site among extranodal lymphomas was GIT.

IHC was performed for sub-classifying the lymphomas. Amongst NHL, the maximum cases comprised B-cell NHL (n=43/56, 76.8%), the rest were T-cell NHL (n=13/56, 23.2%).

Among B-cell NHL, DLBCL comprised the maximum cases accounting for 67.4% (29/43) while anaplastic large cell lymphoma (ALCL) comprised the maximum cases among T NHL accounting for 46.1% (6/13) cases. ((Table/Fig 2)

The 6 cases of HL showed male to female ratio of 2:1 (M=4, F=2). The age ranged from 17 to 63 years with a mean age of 35. All of them were nodal lymphomas and

histologically classified as classic Hodgkin lymphoma. Of these, 3 were mixed cellularity and the other 3 were nodular sclerosis. IHC using LCA, CD3, CD20, PAX-5, CD30, CD15 and EBV (LMP-1) was performed for confirmation.

Table 1: Extranodal sites of involvement by NHL

Site	No. and percentage (N=18)
GIT	7 (38.8%)
Soft Tissue	3 (16.6%)
Tonsil	2 (11.1%)
Nasopharynx	2 (11.1%)
Lung	1 (5.5%)
Liver	1 (5.5%)
Spleen	1 (5.5%)
Vertebrae	1 (5.5%)

Table 2: Sub-classification of Non-Hodgkin lymphomas by IHC

Lymphomas sub-classified by IHC	No. and percentage of patients (n=56)
Diffuse large cell B lymphoma	29 (51.7%)
Anaplastic large cell lymphoma	06 (10.9%)
T-cell lymphoblastic lymphoma (T-LBL)	04 (7.2%)
Follicular lymphoma	04 (7.2%)
Mantle cell lymphoma	03 (5.4%)
Burkitt's lymphoma	02(1.8%)
Small cell lymphoma	01 (1.8%)
Angioimmunoblastic T cell lymphoma	01 (1.8%)
Splenic marginal zone lymphoma	01 (1.8%)
Others*	
NHL-B cell type	03 (5.4%)
NHL-T cell type	02 (3.6%)
* Five cases were broadly classified into B or T cell type but not further classified.	

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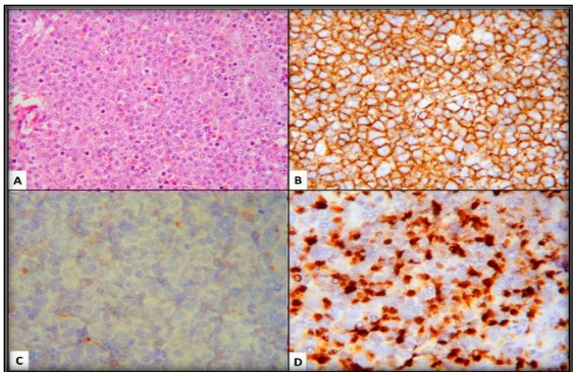

Figure 3: A) Lymph node, Diffuse large B cell lymphoma, Non GCB type. (H & E stain, 400X). B-D) Immunohistochemistry. (B) CD20 positive. (C) CD10 negative. (D)Ki 67- high

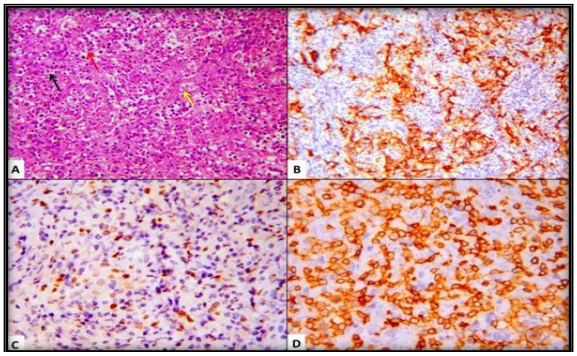

Figure 4. A) Lymph node, Angioimmunoblastic T cell lymphoma showing clear cells (black arrow), high endothelial venules (yellow arrow) and eosinophils (red arrow). H & E stain, 400X. B-D) Immunohistochemistry. (B) CD23 positive (highlight expanded follicular dendritic cell network). (C) Bcl 6 weak positive. (D) CD 3 positive

DISCUSSION

NHL comprised most of the cases (90.3%) while HL comprised 9.7% of cases. Shahid et al in their study found 75% NHL and 25% HL while Sharma M et al found 61 % NHL and 39% HL in their respective studies.[6,7]

Age and gender distribution were comparable to other studies. We noted that among NHL, there was male predominance, similar to findings by Nair R et al. and Shahid et al respectively.[8,6] Mean age of NHL in our study was 55 years with maximum cases in the age group of 50 to 70 years. Neeravari et al found maximum NHL cases in the age group of 50-60 years.[9] Potti et al in their study found maximum NHL cases in the younger age group of 41-50 years.[10] In the present study, within the NHL subgroup, B-cell NHL comprised 76.8% (43/56) while T-cell NHL comprised 23.2%(13/56). Neeravari et al found 80.3% B-cell NHL and 19.6% T-cell NHL while Yasmin et al reported 78.3% B-cell NHL and 21.7% T-cell NHL.[9,11] Within the B-cell category, DLBCL comprised maximum cases accounting for 67.4% (29/43).Table/fig 3 show microscopy and IHC in DLBCL. Shahid et al, Potti R et al and Distano et al also found maximum cases of DLBCL in their study.[6,10,12] Lymphoma diagnosis begins with knowing the relevant clinical and radiological findings followed by morphological assessment of the H & E stained section. This includes architectural alterations and the morphology of lymphoid cells.

Morphologic assessment should take into account the presence of any abnormal population (polymorphic or monomorphic), the determination of pattern (diffuse or nodular) and cell size (small, intermediate, large), and nuclear characteristics (round, irregular, cleaved with condensed or dispersed or blastic chromatin and the character of the nucleoli). [4]

Based on the differential diagnosis, a panel of IHC is planned next. Immunohistochemistry helps to differentiate between subtypes of lymphomas on the basis of their distinctive immunophenotype. The recent development of novel antibodies and upcoming IHC have helped in improving the perfection and accuracy of lymphoma classification.

If the morphology is that of small or intermediate-sized cells affecting B-cell areas, the IHC panel should include CD3, CD5, CD20, Cyclin D1, CD10, CD23, Bcl-2, and

Bcl-6.[13,4] In our study 4 follicular lymphomas, 3 Mantle cell lymphomas, and 1 small cell lymphomas could be subtyped using these markers. When morphology is that of large lymphoid cells, centroblasts or immunoblastic, IHC panel should include CD3, CD20, CD10, BCL6, MUM1, Ki67, BCL2, c-myc and CD30 with optional markers of CD79a, PAX5, CD23, ALK, CD138. This will help confirm a diagnosis of DLBCL and further subclassify it according to Han's algorithm. [14] 'Double Expressor' lymphomas can be identified using BCL2 and c-myc IHC. These markers helped to diagnose all the cases of DLBCL in our study. If T-cell areas are affected antibodies against T-cells may be included like CD2, CD4, CD5, CD8 and CD7.[13]

Immature morphology of intermediate cells with diffuse pattern should prompt an IHC panel of CD99, TdT in addition to CD20, CD 3 CD10 & Ki67 to differentiate B/T lymphoblastic lymphoma and Burkitt lymphoma.[4]

In our study, 4 cases of T-lymphoblastic lymphoma and 2 cases of Burkitt's lymphoma were diagnosed using these markers.

If large anaplastic or RS-like cells are present, the IHC panel should include CD45, CD15, CD30, EBV (LMP-1), CD3, CD20, and PAX5. If required OCT2 and BOB1 may be added. This panel will rule in or rule out Hodgkin lymphoma [14]. In our study, this panel of IHC helped in confirming all cases of HL.

If not Hodgkin lymphoma, then ALCL would be a possibility and IHC antibodies against ALK-1, CD2, CD5, and CD7 may be added.[14] In our study, 6 cases of ALCL were diagnosed using these markers (Table/fig 4). Accurate classification of lymphomas with the help of IHC is very essential for proper treatment and management. Targeted therapies are used based on positive antibodies like Rituximab

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(Anti CD20) for B-cell lymphomas, CD22 – IgG 1 antibody (Epratuzumab) for relapsed NHL, anti-CD30 (SGN 30) for Hodgkin lymphoma, Anti CD40 (SGN 40) in recurrent B-cell NHL and Anti CD80 (Galiximab) for relapsed Follicular lymphoma.[4] Despite its many advantages, there are some limitations to the use of IHC in the classification of lymphomas. The interpretation of immunohistochemical results can be subjective, and there can be variability in the expression of antigens between different lymphoma subtypes. Therefore, it is essential to use a panel of antibodies and to interpret the results in the context of clinical and morphological findings.

CONCLUSION

IHC has emerged as a sensitive diagnostic tool and the most valuable adjunct to H & E staining for accurate sub classification of lymphomas. It is important to remember that careful histopathological examination and clinical correlation remain the cornerstone of morphologic diagnosis which guides IHC. The present study reaffirms the fact that immunohistochemistry is an essential requirement for any standard histopathology laboratory since it plays a vital role in the accurate sub-classification of lymphomas which is essential for therapeutic decision-making. The minimum set of IHC markers required should include included CD45, CD3, CD4, CD8, CD5, CD7, CD10, CD20, CD21, CD23, PAX-5, CD79a, CD15, CD30, BCL2, BCL6, OCT2, MUM1, cyclin D1, c-MYC, Ki67, Alk-1, EBV(LMP-1), CD34, TdT, CD99, CD138, kappa and lambda light chain.

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