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Original Research Article

Spectrum of histopathological diagnosis of lymph node biopsies and utility of immunohistochemistry in diagnosis of lymphoma: A 5 year retrospective study from a tertiary care Centre in South India

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Abstract

Introduction: The lymph node biopsy plays an important role in establishing the cause of lymphadenopathy. The distribution of lymphomas is varied in different geographic regions. The 2016 revision World Health Organization classification of lymphoid neoplasms is used to classify lymphomas.

Aims: To study retrospectively the spectrum of histopathological diagnosis in lymph nodes at our institution, diagnostic utility of immunohistochemical markers in the subtyping of both nodal and extranodal lymphomas and to correlate the histopathology and immunohistochemistry.

Materials and Methods: We retrospectively reviewed lymph nodes between 2012 and 2017, analysed the distribution of cases and classified lymphoma according to the revised 2016 World Health Organization classification of lymphoid neoplasms and compared with other studies.

Results: The distribution of various cases from 235 lymph nodes were as follows: 27% of granulomatous lymphadenitis, 25% of reactive lymphoid hyperplasia, 24% of metastatic deposit, 17% of lymphoma, and 7% of miscellaneous category that included progressive transformation of germinal centres, Kimuras disease, extramedullary hematopoiesis, kikuchi lymphadenitis, neuroendocrine tumor, schwannoma. B-cell lymphomas outnumbered T- cell lymphomas. The statistical parameters were compared between histopathology and immunohistochemistry. Sensitivity, specificity, diagnostic accuracy, positive predictive value and negative predictive value were calculated.

Conclusions: Lymph node biopsy helps in establishing the cause of lymph node enlargement. The distribution of lymphomas in India is varied when compared to the rest of the world. Immunohistochemistry plays an important role in subtyping lymphomas.

Introduction

Lymphadenopathy is a common clinical problem and biopsies confirm whether the etiology is neoplastic or non-neoplastic. The neoplastic disorders are mainly lymphohematogeneous and metastasis while non-neoplastic lesions are varied including infection, reactive changes and miscellaneous disorders like kikuchi Fugimoto disease, Kimura disease, progressive transformation of germinal centre, extramedullary hematopoiesis.[1] Clinically lymphadenopathy can be peripheral or visceral. Lymphoma is a heterogeneous group of lymphoproliferative malignancies with clonal expansion of hematolymphoid cells that share the histologic pattern and immunohistochemistry of their normal counterpart. Diagnosis of lymphoid neoplasms has changed during the last three decades. The World Health Organization classification of tumors of hematopoietic and lymphoid tissues was published in 2001, updated in 2008 and revised in 2016. The revised 2016 WHO updates the current entities and facilitates the recognition of uncommon diseases thus having diagnostic, prognostic and therapeutic implications.[2] In lymphoma panel there is no antibody that is specific and hence the interpretation is based on the panel and knowledge of particular antigen expression in normal, reactive and neoplastic conditions. Correct diagnosis and classification of lymphoma depends on the morphologic, immunophenotypic and molecular genetic features.[17-20] Current study was undertaken to evaluate the spectrum of histopathological diagnosis of lymph node biopsies, ascertain the frequency of Non-Hodgkin lymphoma subtypes and Hodgkin lymphoma subtypes in nodal and extranodal sites based on the WHO classification system by immunohistochemistry, the utility of immunohistochemical markers to subtype lymphomas and to correlate histopathology and immunohistochemistry findings.

Materials and Methods

A retrospective study of all lymph nodes received between 2012-2017 were studied and classified into infective, reactive, metastatic deposit, lymphoma and miscellaneous

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category that included progressive transformation of germinal centres, Kimura disease, extramedullary hematopoiesis, kikuchi lymphadenitis, neuroendocrine tumor, schwannoma. A concise clinical history, examination, and details of relevant investigations were also obtained. These were helpful in reaching a probable clinical diagnosis and formulations of the pathological diagnosis. The data included 235 nodes and the sections were processed using standard methods and stained with Hematoxylin and Eosin stains. The panel of antibodies used for immunohistochemistry included monoclonal antibody to CD2, CD5, CD7, CD10, CD15, CD19, CD20, CD22, CD23, Cd30, CD45, Cd45RO, CD56, Cd68, Cd117, CD1a, bcl2, bcl-6, cyclin-D1, Ki-67, Mum1, FoxP1, SOX11, Alk-1 and polyclonal antibody to Cd3 and Tdt (Table 5). IHC was performed according to avidin-biotin peroxide complex method after pretreatment of antigen retrieval by heating in microwave oven in 0.01M citrate buffer, PH 6.0. All lymphomas were classified according to the standard World health Organization classification of hemato-lymphoid malignancies, 2016. Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy were calculated using immunohistochemistry as gold standard. We could assess the efficacy of immunohistochemistry for categorization of lymphoma and compare it with other studies (Table 4, 5). These statistical parameters were compared between histopathological parameters and immunohistochemistry. The statistical analysis was done using Dx test software (Table 6).

Results

Two hundred and thirty five cases were studied and data included 65 cases (27%) of granulomatous lymphadenitis, 58 cases (25%) of reactive lymphoid hyperplasia, 56 cases (24% of metastatic deposit, 40 cases (17%) of lymphoma, and 16 cases (7%) of miscellaneous (Table 1). Of 40 cases of lymphoma, 27(68%) were non-Hodgkin lymphoma (NHL) and 13 (32%) were Hodgkin lymphoma (HL) (Fig. 1,2). Of 27 NHL cases 20 cases were nodal and the remaining 7 were extranodal (Fig. 3,4). B cell lymphomas (22 cases; 81%) outnumbered T cell lymphoma (5 cases; 19%) (Fig. 5,6). Cervical nodes were the most common site of biopsy (7 cases). The female to male ratio was 2.4:1 (Table 2). Majority of NHL were in 41-50 years of age group followed by 51-60 years of age. The median age is 48 years. Distribution of lesions in female and male patients in different age group and comparative study with other series is shown in table 3, 4. The diagnostic accuracy of H&E was 91% with sensitivity of 92.9% and specificity of 50%. The positive and negative predictive values were 97.5% and 25% respectively. Three cases did not correlate with immunohistochemistry and the discordant cases were kikuchi lymphadenitis, reactive lymphoid hyperplasia and progressive transformation of germinal centres. Out of 13 cases of Hodgkins lymphoma classical type was the most common subtype comprising 61% (8 cases) of cases of which 6 were EBV positive and 2 EBV negative. It was followed by 30% (4 cases) of nodular lymphocyte predominant type of which 3cases were EBV positive and 1 EBV negative. There was one case of mixed cellularity Hodgkin lymphoma that was EBV positive. Metastasis constituted the remaining malignancies representing 24% of total lymph node biopsies. Out of 56 cases of metastatic lymphadenopathies, 22% (12 cases) were squamous cell carcinoma, 66% (37 cases) were adenocarcinoma, 5% (3 cases)were poorly differentiated carcinoma, 5%(3 cases) of papillary carcinoma thyroid and 2% (1 case) of small cell carcinoma.

Table 1: Distribution of diagnosis in lymph nodes (n=235)

S. No.	Diagnosis	Number of cases
1.	Granulomatous lymphadenitis	65
2.	Reactive lymphoid hyperplasia	58
3.	Metastatic deposit	56
4.	Lymphoma	40
5.	Suppurative lymphadenitis	4
6.	Non-specific lymphadenitis	3
7.	Kimura disease	2
8.	Progressive transformation of germinal centres	1
9.	Extramedullary hematopoiesis	1
10.	Kikuchi lymphadenitis	3
11.	Schwannoma	1
12.	Neuroendocrine tumor	1

Table 2: Demography of lymphomas

Non-Hodgkin lymphoma(n=27)			Hodgkin lymphoma(n=13)	
Age(years)	**Males**	**Females**	**Males**	**Females**
<10	1	0	0	1
11-20	2	0	3	0
21-30	1	0	0	2
31-40	2	1	0	0
41-50	6	2	2	0
51-60	4	2	1	1
61-70	2	2	2	1
71-80	1	1		

Table 3: Incidence of B- cell and T-cell lymphomas in various studies

Diagnostic category	Present study	Rao A et al(2013)	Padhi S et al(2012)	Mushtaq S et al(2005)	Kalyan K et al(2006)	Naresh K N et al(2000)	Sharma M et al(2014)	Howell et al(2012)
B cell lymphoma	81%	54%	96%	86%	72%	79.1%	89.3%	98%
T-cell lymphoma	19%	38%	4%	24%	67%	16.2%	10.7%	2%

Table 4: Distribution of B-cell lymphomas in various studies

Diagnostic category	Present study	Rao A et al(2013)	Padhi S et al(2012)	Mushtaq S et al(2005)	Kalyan K et al(2006)	Naresh K N et al(2000)	Sharma M et al(2014)	Howell et al(2012)
DLBCL	56%	29.3%	69%	76%	26%	34%	46.8%	47%
FL	21%	6.8%	8%	6%	4%	12.6%	12.8%	8%
MCL	4%	4%	5%	4%	3%	3.4%	17%	5%

Table 5: Immunohistochemical distinction between DLBCL and HL

IHC markers	DLBCL	HL
CD30	-/+	+(Uniform)
CD15	-	+
Cd45	+	-
PAX5	+(strong)	+(weak)
CD20	+(strong,uniform)	-/+(weak)
EBER	-	+
OCT2/BOB1	+	-
CD79a	+	-
Bcl6	-	-
P-63	+	-

Table 6: Point estimates and 95% confidence intervals

Parameters	Point estimates (95%CI)
Sensitivity	92.9%(80.5-98.5)
Specificity	50%(1.3-98.7)
Positive predictive value	97.5%(86.8-99.9)
Negative predictive value	25%(0.6-80.6)
Positive likelihood ratio	1.857(0.463-7.445)
Negative likelihood ratio	0.143(0.024-0.833)
Odds ratio	13(0.641-263.815)

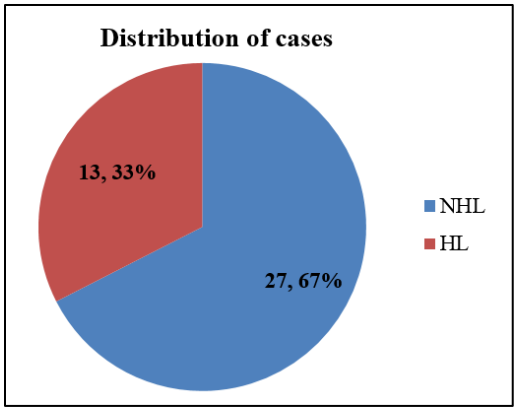

Fig. 1: Chart depicting distribution of lymphomas

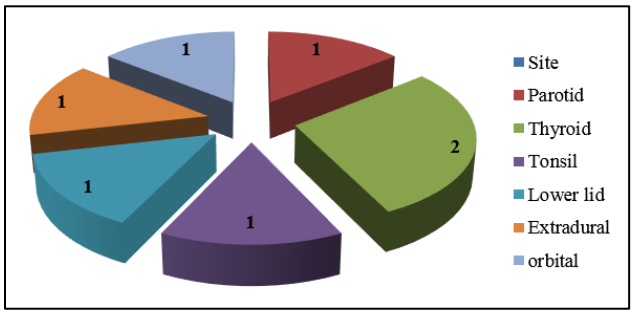

Fig. 2: Chart depicting extra nodal lymphomas

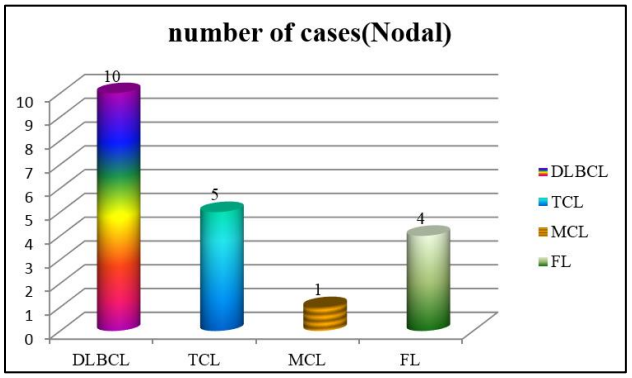

Fig. 3: Chart depicting subtypes of non-Hodgkin lymphoma in different nodes

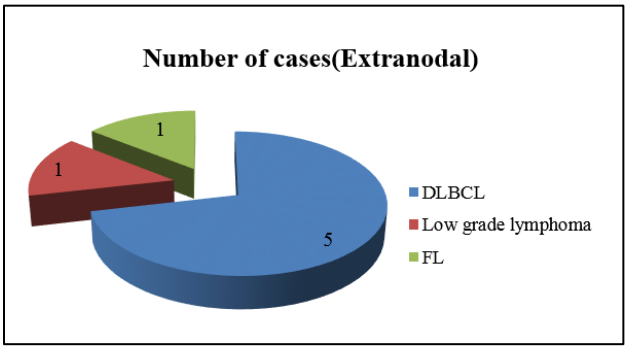

Fig. 4: Chart depicting subtypes of non-Hodgkin lymphoma in extra nodal sit

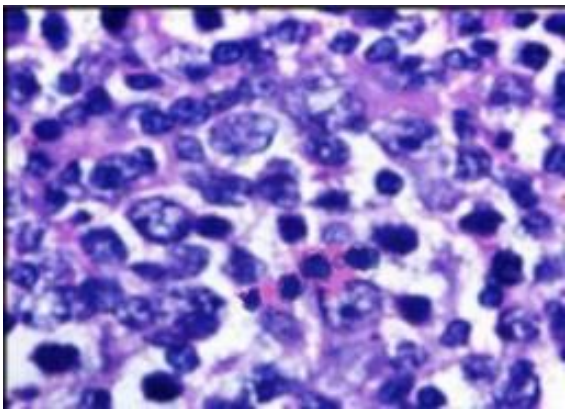

Fig. 5: Microphotograph of Diffuse large B-Cell lymphoma (H&E x200)

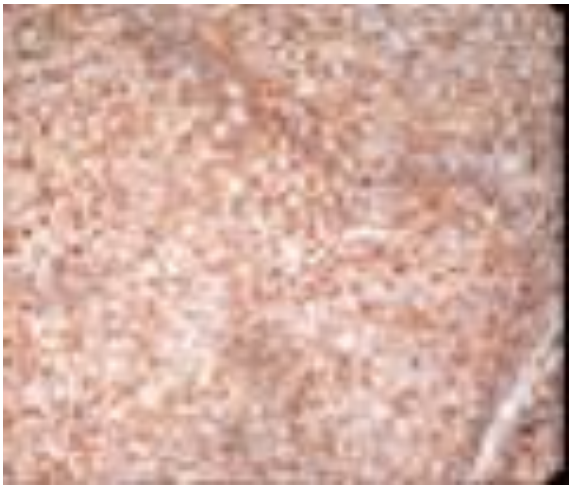

Fig. 6: Microphotograph of tumor cells positive for CD20 (IHC x200)

Discussion

The present study showed granulomatous lymphadenitis was the most common cause of lymphadenopathy (27%) followed by reactive lymphoid hyperplasia (25%), metastatic deposit (24%), lymphoma (17%) and miscellaneous causes (7%) Lymphoma is a group of lymphoproliferative malignancies with distinct causes, patterns of behavior and responses to treatment. Approximately 30% of NHL arise from site other than lymph node, spleen or bone marrow.[21-24] Site of localization and histologic type are the most important predictor of prognosis in extranodal lymphomas. Immunohistochemistry is useful and necessary diagnostic test that helps in subtyping different types of lymphoma.[10-16] Age and gender are the risk factors for NHL and affect the treatment outcome of the disease and survival rate of patients. The occurrence of NHL is higher in men (worldwide age-standardised rate-ASR 6.1/100000) than women (ASR 4.2/100000). In our study also male to female ratio is 2.4:1. NHL is more common in older than young patients. In our study NHL was seen in age group 41-50 years (22%) followed by 51-60years (15%). The median age in our study is 48 years. We compared our results with Rao et al,[1]

Vallabhajosyula et al and Padhi et al (Table 4, 5). Rao et al reported 63.6% NHL occurred after 40 years of age with peak between 51-60 years. The median age of study population was 55.5 years in a study conducted by Vallabhajosyula et al.[3] Padhi et al[4] too reported the peak incidence during 4th to 5th decade. In our study B-cell lymphomas outnumbered T-cell lymphoma and the most common subtype being diffuse large B Cell lymphoma (DLBCL), followed by Follicular lymphoma, mantle cell lymphoma. We compared our results with Sharma et al,[5] Rao et al,[1] Kalyan et al,[6] Padhi et al,[4] Howell et al,[7] Naresh et al[8] and Mushtaq et al[9] (Table 4,5). Out of 13 cases of Hodgkins lymphoma classical type was the most common subtype comprising 61% (8 cases) of cases of which 6 were EBV positive and 2 EBV negative. In our study, the diagnostic accuracy of H&E was 91% with sensitivity of 92.9% and specificity of 50%. The positive and negative predictive values were 97.5% and 25% respectively. Three cases did not correlate with immunohistochemistry and the discordant cases were kikuchi lymphadenitis, reactive lymphoid hyperplasia and progressive transformation of germinal centres.

Conclusion

Lymph node biopsy plays an important role in establishing the cause of lymphadenopathy. The distribution of lymphoma is varied and is comparable with the rest of the world. Age, gender and histologic subtype have prognostic and theranostic implications. Meticulous morphologic evaluation and a concise immunohistochemical panel is mandatory in subtyping nodal and extranodal lymphomas.

Future Studies

More number of cases need to be studied in future as the pathological and molecular heterogeneity of DLBCL has implications in molecular targeted and personalized therapy.

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Conflict of Interest: None.

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