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Original Research Article

Morphological and Immunohistochemical Categorization of Malignant Lymphomas

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Abstract

Lymphomas are broadly classified in to Hodgkins lymphoma (HL) and Non Hodgkins lymphoma. Immunohistochemistry helps in better understanding of pathogenesis and also useful in the characterization of immunophenotype in most of the malignant lymphomas. *Aims & Objectives:* The current study was undertaken to categorize malignant lymphomas based on morphological and immunohistochemical findings. *Materials and Methods:* Fifty cases of Lymphomas were diagnosed in the Department of Pathology, at MVJ Medical College Research Hospital, over a period of two year (between April 2015 and April 2017). The data was analyzed with respect to age, sex, histomophological and immunohistochemical characters. *Results:* Out of 50 cases, 12 were of HL (24%) and 38 cases (76%) were NHL. Among NHL, B-cell and T-cell lymphomas were of equal incidence. Male's outnumbered females (68%) & maximum incidence of lymphomas including both sexes was seen in age group 51-60 years followed by age group 41–50 years. *Conclusion:* IHC is a useful adjunct modality with H&E stain in diagnosis and classification of malignant lymphomas.

Keywords: HL; NHL; T-cell; B-cell; RS cell.

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Introduction

The lymphomas are a heterogenous group of malignant lympho-proliferative diseases of lymhnodes or of extranodal lymphoid tissue. They are broadly classified in to Hodgkins lymphoma (HL) and Non Hodgkins lymphoma.

The etiology of lymphomas is largely unknown. Some of the risk factors include severe immunodeficiency, various infectious agents, familial aggregation, blood transfusion, and occupational exposure to pesticides and solvents.[1]

Immunohistochemistry helps in better understanding of pathogenesis and also useful in

the characterization of immunophenotype in most of the lymphomas.[2] Based on histomorphological differential diagnosis, the panel of IHC markers will be decided which includes leukocyte common antigen (LCA), B-cell markers (CD20 and CD19), T-cell markers (CD3 and CD5) other markers like bcl-2, CD15, CD30, CD138, CD68, ALK-1. No single marker is specific for diagnosis of lymphoma hence positivity of any antibody should be interpreted based on the knowledge of antigenic expression in normal, reactive and neoplastic conditions.[3]

Aims & Objectives:

The current study was undertaken to categorize malignant lymphomas based on morphological and immunohistochemical findings.

Materials and Methods

Fifty cases of Lymphomas were diagnosed in the Department of Pathology, at MVJ Medical College & Research Hospital, over a period of two year (between April 2015 and April 2017). Patients were informed about the importance of the study and consent was taken in all the cases. The clinical information regarding age, sex and site of the biopsy were taken into account on routine haematoxylin and eosin stained sections.

Of the total 50 cases diagnosed provisionally as lymphoma on light microscopy, were taken up for immunophenotyping with IHC studies. They were classified based on WHO classification according to the positive or relevant negative immonophenotypic expression and the results tabulated to ascertain the morphological spectrum of lymphomas in this rural area of Hoskote.

IHC Panel employed in the Study

The panel for antibodies used for immunohistochemistry included monoclonal anibodies to LCA, CK, CD3, CD19, CD20, CD15, CD30, CD56, CD138, CD68, CD 45RO, BCL-2, Ki-67, and ALK-1. Immunohistochemistry was performed by avidin-biotin peroxidase complex method, after antigen retrieval by heating in pressure cooker in 0.01M citrate buffer (pH-6.0). The results were tabulated and recorded individually.

Results

Of 50 cases, Cervical Lymph Nodes were most common site of biopsy (26 cases).

Male's outnumbered females as 68% cases were of male patients and 32% were females. Maximum incidence of lymphomas including both sexes was seen in age group 51–60 years followed by age group 41–50 years.

In this study, 12 cases (24%) were of HL and 38 cases (76%) were NHL. Among NHL, B-cell and T-cell lymphomas were of equal incidence (Table 1).

Table 1: Distribution of various lymphomas

Subtypes	Number	Percentage
Hodgkin's lymphoma	12	24
B-cell lymphoma	19	38
T-cell lymphoma	19	38
Total	50	100

Out of 12 cases of Hodgkin's lymphoma, mixed cellularity subtype of classical variant was the most common. Incidence was higher in females compared to males. In all these cases, RS cells were positive for CD30 and CD15 (Table 2).

Table 2: Types of Hodgkin's lymphoma

Type of HL	No of cases	Age in years							
		0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80
1) Nodular lymphocyte predominance	1	1	–	–	–	–	–	–	–
2) Classical									
a. Mixed cellularity	7	–	2	–	–	2	1	1	1
b. Nodular Sclerosis	3	–	–	1	1	–	1	–	–
c. Lymphocyte rich	Nil	–	–	–	–	–	–	–	–
d. Lymphocyte depleted	1	–	–	1	–	–	–	–	–

Among 38 cases of Non Hodgkin's lymphoma, 19 cases were B-cell lymphomas and 19 cases were T-cell lymphomas but incidence was higher in males compared to females. Maximum cases were seen in the age group of 41–60 years. Among B-cell NHL diffuse large B cell lymphoma was the most common and followed by small lymphocytic lymphoma whereas in T-cell NHL, Peripheral T-cell lymphoma, NOS type was most common.

Table 3: Age and sex distribution of NHL

S. No	Age in years	Male		Female		Total	Percentage
		B-cell	T-cell	B-cell	T-cell		
1.	0-10	–	–	–	–	Nil	Nil
2.	11–20	–	1	–	1	2	4
3.	21–30	2	–	1	–	3	6
4.	31–40	3	1	–	–	4	8
5.	41–50	3	6	–	1	10	20
6.	51–60	3	4	2	1	10	20
7.	61–70	4	2	–	–	6	12
8.	71–80	1	2	–	–	3	6

Table 4: Immunohistochemical profile of B-cell lymphomas

Lymphoma Subtypes	No of cases	IHC markers
Small lymphocytic lymphoma	5	CK-, LCA+, CD19+, CD20+.
Lymphoplasmacytic lymphoma	2	CK-, LCA+, CD19+, CD20+, MIB-1+
Follicular lymphoma	3	CK-, LCA+, CD19+, CD20+, BCL2+,
Diffuse large B-cell lymphoma	6	CK-, LCA+, CD19+, CD20+, CD3-, MIB-1+
Plasmacytoma/myeloma	2	CK-, LCA+, CD19+, CD20+, kappa +
T-cell rich B cell lymphoma	1	LCA+, CD19+, CD20+, CD3 (T cells)
Total	19	

Table 5: Immunohistochemical profile of T-cell lymphomas

Lymphoma Subtypes	No of cases	IHC markers
Peripheral T-cell lymphoma, NOS	12	LCA+, CD3+, CD20-
Mycosis fungoides	3	LCA+, CD3+, CD20-
Angioimmunoblastic T-cell lymphoma	1	LCA+, CD3+, CD20-
Anaplastic large cell lymphoma ALK+	1	LCA+, CD3+, CD20-, CD68+, CD15-, CD30+, ALK+.
Anaplastic large cell lymphoma ALK-	1	LCA+, CD3+, CD20-, CD68+, CD15-, CD30+, ALK-.
Enteropathy associated T-cell lymphoma	1	LCA+, CD3+, CD20-
Total	19	

Table 6: Incidence rate of HL and NHL in various studies

Authors name (year)	HL	NHL
Hazra K et al., (2017)[4]	14.2%	85.8%
Sharma M et al., (2014)[5]	39%	61%
Roy A et al., (2013)[6]	27.8%	72.2%
Current Study	24%	76%

Table 7: Incidence rate of B-cell and T-cell lymphomas in various studies

Authors name (year)	B-cell lymphoma	T-cell lymphoma
Sharma M et al., (2014)[5]	89.3%	10.7%
Roy A et al., (2013)[6]	54.0%	38.0%
Padhi S et al., (2012)[7]	96.0%	4.0%
Mushtaq S et al., (2008)[8]	86.0%	24.0%
Current Study	50.0%	50.0%

Table 8: Distribution of Extranodal lymphoma.

S. No.	No of cases	HL	B-cell	T-cell	Total
1.	Testis	–	3	Nil	3
2.	Skin	–	Nil	3	3
3.	Tonsil	–	1	Nil	1
4.	Nasal cavity	–	Nil	1	1
5.	GIT	–	1	3	4
7.	Thyroid	–	1	–	1
Total					13

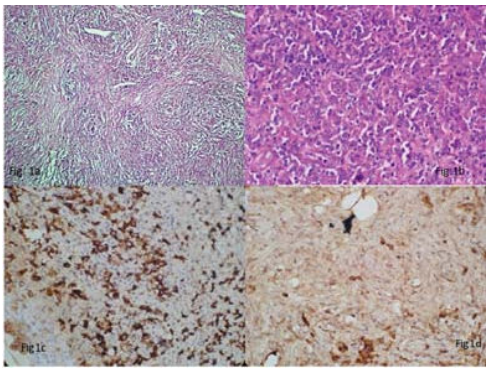

Fig. 1: Hodgkin lymphoma. 1a: Nodular sclerosis (H&E, 100x); 1b: Mixed cellularity (H&E, 400x); 1c: CD15 Positive (IHC, 400x); 1d: CD30 positive (IHC, 400x).

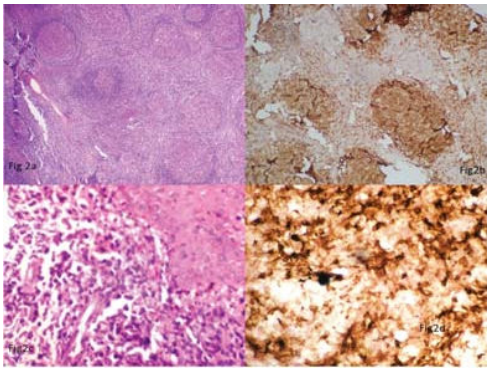

Fig. 2a: Follicular lymphoma (H&E, 100x),: **2b:** Follicular lymphoma wiht CD20 positivity (IHC, 400x): **2c:** Mycosis fungoides (H&E, 400x): **2d:** Mycosis fungoides with CD3 positivity (IHC, 400x).

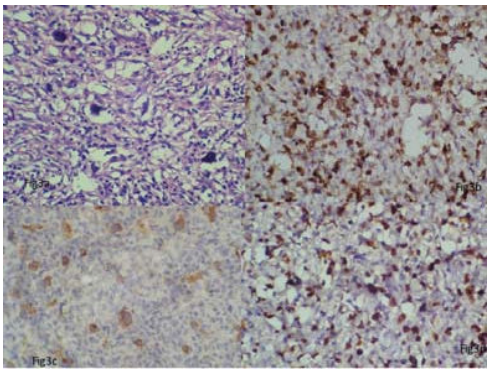

Fig. 3a: T-cell lymphoma (H&E, 100x); **3b:** T-cell lymphoma with CD3 positivity (IHC, 400x); **3c:** Tcell lymphoma with CD30 positivity (IHC, 400x); **3d:** T-cell lymphoma with MIB-1 positivity (IHC, 400x).

Discussion

Immunohistochemistry has become an integral part of diagnostic histopathology and it is very helpful in phenotyping the abnormal lymphoid population completely which were detected by morphology. IHC is also useful in identification of subtle abnormal population in reactive tissue.[3] The incidence of NHL is 3–4 times higher compared to HL in Western countries than in India.[1,9,10] This may be due to racial and genetic factors.

Hodgkins lymphomas are diagnosed morphologically by identifying Reed Sternberg cells in a background of reactive population consisting of reactive lymphocytes, histiocytes, plasma cells, eosinophils, neutrophils and fibroblasts. All these cases were subjected to immunohistochemistry by using following markers CD30, CD15, LCA, CD20, and CD3 for confirmation. In our study, all the cases RS cells were positive for CD30 and CD15. In this study, 7 cases were diagnosed as mixed cellularity, 3 cases as nodular sclerosis and one case each as nodular lymphocytic predominance and lymphocyte depleted type of Hodgkins lymphoma. In our study mixed cellularity subtype is a most common type which is not concordant with Hazra K et al.[4] and Roy A et al.[5] study since in their study nodular sclerosis subtype of CHL is a commonest type.

NHL is more common in older than in younger individuals, hence age may play a stronger risk factor for NHL occurrence. Even in present study maximum number of cases were seen in age group 41–60 years, which is in concordant with the similar study done by Roy A et al., Padhi et al., and Valabhajosyula et al.[6,7,11]

Incidence rate of HL is lower than NHL in our study. Various studies also show similar findings. Among NHL, the prevalence of B-cell and T-cell lymphomas is equal in our study. In other studies B cell lymphomas outnumbered T-cell lymphomas. This could be attributed to geographical variation. There are some evidences which also say that the rate of T-cell lymphomas is higher in Asian countries than western countries.[12]

Extranodal lymphomas account to one-third of non-Hodgkin lymphomas (NHL) approximately and these originate from sites other than lymph nodes, spleen or the bone marrow. Extra nodal lymphomas can arise in almost every organ. In the literature it is shown that gastrointestinal (GI) tract, skin, bone, and brain are the most common sites. Ann Arbor staging system considers tonsils and the Waldeyer's ring as lymphatic localizations, but there is controversy regarding their designation as extranodal sites. If they are included in the extranodal lymphoma category, the head and neck will be the second most frequent site.[13] Even in this study, extra nodal lymphomas contributed 26%.

Among B-cell lymphomas, commonest type is DLBCL with highest cases, which is comparable to other studies.[14] Out of T-cell lymphomas, Peripheral T-cell lymphoma, NOS type is the commonest. In a similar study by Roy et al. also reported highest incidence.[6]

Conclusion

Studies have documented that the overall decline in non–Hodgkin lymphoma incidence, but still T-cell lymphomas continues to rise. Since historically T-cell lymphomas were rare entity now it has emerged as a challenging area to understand the etiology with clinical characteristics, treatment and prognosis. Hence immunophenotyping has become mandatory for the diagnosis and classification of malignant lymphomas and to avoid diagnostic pitfalls. IHC markers study is also useful in predicting the prognosis and in initiation of appropriate therapy.

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