Role of Immunohistochemistry in Differential Diagnosis of Lymphoma (A Study of 200 Cases)

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Abstract

Introduction: The Lymphomas are a heterogeneous group of lympho-proliferative malignancies, with distinct causes and showing distinctive patterns of behaviour and responses to treatment. World Health Organization broadlyclassifies lymphomas into Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). Non-Hodgkin lymphoma is further subclassified based on the stage of maturation and cell of origin [B cell, T cell, or natural killer cell (NK) cell]. The panel of markers is decided based on morphologic differential diagnosis (no single marker is specific) which includes leukocyte common antigen (LCA), B-cell markers (CD20 and CD79a), T-cell markers (CD3 and CD5) and other markers like CD23, bcl-2, CD10, cyclinD1, CD15, CD30, ALK-1, CD138 (based on cyto-architectural pattern).

Aims & Objectives

- To Study Distribution and Frequency of lymphoma by Age and Gender.
- To Study Incidence and Histopathological Grading of HL and NHL in population.
- To Correlate the Histopathological Diagnosis and Immunohistochemistry Diagnosis.
- To Study the Role & Significance of Immunohistochemistry in confirmation of diagnosis and accurate typing of Lymphoma.

Material and Method:

This study was conducted at a tertiary centre in B.J.medical collage, civil hospital & GCRI ,

ahemdabad, asarwa and included a total of 200 cases of lymphoid malignancies . As a part of this study, 200 surgical specimens were received from January 2016 to september 2017. As a part of this study, 200 surgical specimens were received from January 2016 to september 2017. Specimens were studied with keeping the following features in mind: Age, Sex and site of tumours. All age and sex groups were included in this study. Histopathological diagnosis was made on all the sections studied. Immunohistochemistry was done for all these cases and final diagnosis was made.

Result: Amongst the 200 patients enrolled 127 (63.5%) were males and 73 (36.5%) were females. The male to female ratio was 1.73:1. Maximum patients were belonged to the age group of 51-60 years. Non Hodgkins Lymphoma :157(78.5%) is much more common than Hodgkins Lymphoma :43(21.5%). Among NHL Group Diffuse Large B cell Lymphoma is the Predominant type followed by, follicular Lymphoma, Precursor Lymphoblastic Lymphoma. IHC in HL shows CD30 positivity is more as compared to CD15 positivity.

Conclusion: Immunohistochemistry is required for confirmation, Typing, prognosis and Treatment. Single IHC mrker is not sufficient for confirmation of diagnosis. Further, the immunophenotyping of lymphoid tumors is now considered to be vital for better management, prognosis and for routine pathological evaluation of lymphoproliferative disorders.

Keywords*: Lymphoid malignancies, hodgkin disease, non- hodgkin lymphoma, immunohistochemistry.*

Introduction

Malignant lymphoma is the generic term given to the tumors of lymphoid system and their precursor cells whether T, B or null phenotype. The malignant lymphomas can be divided into two major categories, Hodgkin lymphomas characterized by Reed – Sternberg (R– S cells) and all others collectively known as Non – Hodgkin's lymphoma. The diagnosis of malignant lymphomas until recently was primarily based on morphology. However, the advent of immunohistochemistry has revolutionized the diagnosis of lymphoma and serves an important diagnostic tool in the current WHO classification [1]. In the present scenario interpretation of morphological features together with immunohistochemistry has become the bedrock for diagnosis and therapy of lymphoid malignancies. These studies have not only provided the objective classification but have identified the antigens that can be targeted for therapy [2]. All lymphomas were classified either as HD (Hodgkin's disease) or NHL (Non– Hodgkin's lymphoma) and further categorization was done using WHO classification [3].

Morphological identification and classification of lymphomas based on immunophenotyping is of paramount importance for the management of patients and determining the prognosis because each type and subtype exhibits distinct clinicopatholgic features. The pathological classification of lymphoid malignancies has been a controversial subject. Numerous classifications were proposed used and then went out of favor because there were always lacunae that defied comprehension. Repeated attempts for a unified classification were complicated by extra-ordinary confusion of terminology[4].

Application of monoclonal antibodies and subsequent immunophenotyping is now accepted worldwide. It serves a necessary adjunct to the morphological diagnosis of Leukemia and lymphomas. The recent REAL classification and WHO classification are primarily based on morphological and immunohistochemical characteristics [5,6].

Diagnosis of HD is made by the identification of RS cells in an appropriate reactive milieu comprising small lymphocytes, histiocytes, epithelioid histiocytes, neutrophils, eosinophils, plasma cells and fibroblasts in different proportions. In most of the cases, HD can be diagnosed morphologically. However, application of immunohistochemestry has enabled the recognition of subgroup that has led to the modification of previous Rye classification with the recent WHO classification [7,8].

Material and Method

As a part of this study, 200 surgical specimens were received from January 2016 to january 2019, in Tertiary care hospital. The gross examination of the specimens was done. The following points were noted regarding size, shape, consistency and outer appearance. 5-micron thick sections were cut from 10 % neutral buffered formalin fixed and paraffin embedded tissues. Routine haematoxylin and eosin (H & E) staining were employed for all the section studied. Special stains were done as and when required. Histopathological diagnosis was made on all the sections studied. Immunohistochemistry was done for all these cases and final diagnosis was made.

Immunohistochemical staining was performed on BENCHMARK XT(IHC/ISH) by automated immunostainer.

The panel of monoclonal antibodies routinely used included CD45, CD20, CD3, CD15 and CD30 (BIOGENEX). In specific cases antibodies against epithelial membrane antigen (EMA), cytokeratin and S – 100 were used. The cases that were diagnosed as Round cell malignancy on morphology but after special stain and immunostaining did not fall into category of lymphoma were excluded from the study. All lymphomas were classified either as HD (Hodgkin's disease) or NHL (Non– Hodgkin's lymphoma) and further categorization was done using WHO classification. Also, International Working Formulation was employed and NHL's were subdivided into high, intermediate and low grade.

Ethical Considerations: All procedures performed were in accordance with the ethical standards of the institution.

Observation and Result

TABLE -1 Incidence of HL AND NHL (N=200)

Serial No.	Diagnosis	No.of Cases	Percentage
1	Hodgkin lymphoma	43	21.55%
2	Non Hodgkin lymphoma	157	78.5%
	Total	200	100%

My study shows 43 (21.55%) cases of Hodgkin's lymphoma and 157 (78.5%)cases of Non Hodgkin's lymphoma.

Table -2 Site Wise Distribution of Lymphoma (N=200)

No.	Site	TOTAL cases	HL	NHL
1	Nodal lymphoma			
	Cervical	68	28	40
	Axillary	19	5	14
	Inguinal	12	2	10
	iliac/sacroiliac	5	2	3
	Submandibular	5	2	3
	Mesenteric	7	-	7
	Mediastinal	5	1	4
	Submental	1	-	1
	Paraaortic	3	1	2
	Supraclavicular	3	2	1
	Abdominal node	5	-	5
	Total	133	43	90
2	Extranodal lymphoma			
	GIT	14		
	Bone	5		
	Bone marrow	6		
	soft tissue mass	12		
	Spleen	3		
	Liver	4		
	Parotid	4		
	Testis	3		
	nasal/nasopharynx	8		
	Thyroid	2		
	Brain	6		
	Total	**67**		**67**

As the Lymphoma is basically a Disease of lymphoid system but there are many cases which develops extra nodal lymphomas.In our study there are 90(57.32%) nodal NHL and 67(42.68%) extra nodal NHL which suggest Nodal NHL is common than Extra nodal NHL.

TABLE -3 AGE AND SEX WISE INCIDENCE RATE OF STUDY (N=200)

Sr.No.	Age Group(year)	Male	Female	Total	(%)
1	<10	6	6	12	6
2	11-20	21	9	30	15
3	21-30	9	4	13	6.5
4	31-40	17	7	24	12
5	41-50	19	16	35	17.5
6	51-60	28	20	48	24
7	61-70	18	10	28	14
8	71-80	9	1	10	5
	TOTAL	127	73	200	100%

The incidence of lymphoma was higher in males as compared to female. The age distribution ranging from 51-60 yrs (most common) and 11-20 years (2nd most common). Bimodal age of distribution may be due to relative immune deficient status in this age group. Male:Female ratio is 1.73:1 for all lymphomas in this study.

Discussion

Table – 4 Distribution Of Types Of Hodgkin's Lymphoma (N=43)

Sr. No.	Hl Type	No.of Cases	%
1	Classical HL	41	95.30
2	Nodular lymphocyte predominant hodgking lymphoma	2	4.70
	TOTAL	43	100

Site wise distribution in Hodgkins lymphoma shows only Nodal involvement. No extranodal HL has found in our study. Most common involvement is cervical lymphnode followed by axillary ,supraclavicular and submandibular region. In extranodal NHL most common site for involvement is GIT followed by soft tissue involvement ,nasal/ nasopharynx,brain,bone.

Table -5 Distribution of Types of Nhl According to Who Classification (N=157)

Sr.no	Nhl Type	No.of Cases	Percentage
B cell Lymphomas			
1	Diffuse large B cell Lymphoma	60	38.21%
2	Follicular lymphoma	17	10.82%
3	Mantle cell lymphoma	2	1.27%
4	Precursor/Lymphoblastic	16	10.19%
5	T cell rich B cell Lymphoma	3	1.91%
6	Burkitt's Lymphoma	7	4.45%
7	Small lymphocytic Lymphoma	9	5.73%
8	Plasmacytoma	5	3.18%
9	Splenic marginal zone Lymphoma	2	1.27%
10	CD20+ B cell lymphoma	15	9.54%
	TOTAL	**136**	
	T cell Lymphomas		
1	Precursor	8	5.09%
2	Anaplastic Large Cell Lymphoma	5	3.18%
3	PTCL	4	2.54%
4	Mycosis fungoidus(cutaneous T cell lymphoma)	1	0.63%
5	Others	3	1.91%
	TOTAL	**21**	

Table : 6 IHC panel for differential diagnosis of lymphoma

Sr.No	FINAL DIAGNOSIS	IHC PANEL
1	HL CLASSICAL TYPE	CD 15 positive CD30 positive
2	Nodular lymphocytic predominant	CD 20 positive along with CD 15,CD 30 negative
	B cell neoplasm	
1	DLBCL	LCA-positive AE1 - negative CD20-Positive
2	Follicular lymphoma	CD20-s-Positive MIB-I-Positive in 85 % cells BCL 2 –positive CD20-Positive CD10-positive
3	Mantle cell lymphoma	Combined positivity for CD 20,CD 5,Cyclin D1
4	T cell rich B cell Lymphoma	CD20(Strong Positive) LCA (Strong Positive) AE1 - negative Vim – inconclusive
5	Burkitt's Lymphoma	CD 20,MIB 1 :positive Few case LCA : positive CD 10: negative
6	Small lymphocytic Lymphoma	CD 5, CD 23 ,CD20 : positive Few case CD 10 positivity
7	Plasmacytoma	CD 38, CD 138 with MUM 1 :positive
8	Splenic marginal zone Lymphoma	CD20,CD23 : positive
	T cell neoplasm	
1	Precursor T celllymphoma	CD2,CD 79a MIB 1 :positive Few case CD 3 positive
2	Anaplastic large cell lymphoma	CD 2,CD 30 :positive
3	Peripheral T cell lymphoma	CD 2,CD 30,LCA :positive
4	Mycosis fungoids	CD2, CD3, CD 30 :positive

In present study we can say that IHC is the Final Diagnostic tool for Confirmation of diagnosis.There are 17 cases in which Diagnosis on Histopathological examination is different from IHC diagnosis. Out of those 17 cases, 3 cases show p/o malignant round cell tumor on Histopathological examination which on IHC show confirm diagnosis of B cell NHL. In all these 3 cases Both CD20 And LCA are positive which suggest B cell NHL. 2 cases show p/o poorly diiferentiated malignant tumor on Histopathological examination which on IHC shows confirm diagnosis of B cell NHL. In these 2 cases there is also CD 20 And LCA both are Positive which shows B cell NHL. 2 cases show p/o anaplastic large cell lymphoma on Histopathological examination which on IHC show confirm diagnosis of Hodgkin Lymphoma. In these 1case show CD30 positive along with CD20 and LCA positivity which confirms diagnosis of HL. Other case show CD30 & CD15 positive, which confirm the diagnosis of HL.

Conclusion

Amongst the 200 patients enrolled 127 (63.5%) were males and 73 (36.5%) were females. The male to female ratio was 1.73:1. Maximum patients were belonged to the age group of 51-60 years. Non Hodgkins Lymphoma 157(78.5%) is much more common than Hodgkins Lymphoma43(21.5%). Non Hodgkins Lymphoma shows B cell NHL is more common than T cell Lymphoma. Among NHL Group Diffuse Large B cell Lymphoma is the Predominant type followed by, follicular Lymphoma ,Precursor Lymphoblastic Lymphoma. In NHL,Nodal involvement is more common than extranodal NHL. Cervical Lymphnode involvement is the most comon site in Both NHL and HL. No HL case has shown Extranodal involvement. In Extranodal NHL, GIT is most common site of involvement. IHC in HL shows CD30 positivity is more as compared to CD15 positivity. In DLBCL,IHC shows important role of BCL2 along with CD20 AND LCA. In Follicular Lymphoma,combined positivity of CD20 ,CD10 and BCL 2 has diagnostic role. CD20 positive B cell lymphoma is the broad category which include all B cell NHL with CD20 Positivity. Immunohistochemistry Has been done for all this 200 cases. From which 17 cases the diagnosis has been changed/Confirmed according to marker Expression.

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