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Histopathological and Immunohistochemical Analysis of Lymphoma: Study from a Tertiary Care Centre

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ABSTRACT

Background- Lymphomas are malignant lymphoproliferative diseases, broadly classified by WHO into Hodgkin Lymphoma (HL) & Non-Hodgkin Lymphoma (NHL). HL should be segregated from NHL group, as clinically and histopathologically both are distinct entities, the prognosis & treatment of which varies.

Material and methods- A retrospective study was conducted in S.C.B Medical College, Cuttack, a tertiary care centre in Odisha to correlate histomorphological & immune -histochemical findings and subsequent subtyping of lymphoma according to WHO classification. 92 cases of lymphoma were diagnosed between January 2014 to February 2017 for a period of 3 years and correlation was done with respect to age, sex, histomorphological and immunohistochemical findings.

Result- Out of 92 cases of lymphoma, 13 were HL (14.2%), 79 cases were NHL (85.8%). Among all NHL cases extra-nodal lymphoma constituted 28(35.4%). In NHL Male to Female ratio was 1.8:1 and maximum number of cases presented between 4th to 5th decades. Diffuse large B cell lymphoma was the most common type of NHL and Nodular sclerosis was the commonest type of HL.

Conclusion- Conventional H&E stain in adjunct with immunohistochemistry was useful modality in diagnosis and classification of lymphomas.

Introduction

Lymphoma is a group of malignant lymphoproliferative disease of lymphnodes or other lymphoid organs in extranodal sites. Lymphoma is the 7th most common type of malignancy in both sexes. It is broadly divided in to Hodgkins Lymphoma(HL) and Non-Hodgkins Lymphoma(NHL). Lymphnode involvement is seen in HL and 2/3rd cases of NHL where as in rest 1/3rd cases extranodal sites are involved. NHL can be T-cell, B-cell or NK cell type. Histomorphologically HL is characterized by presence of Reed-Sternberg giant cells mixed with reactive lymphocytes, histiocytes, plasma cells, eosinophils & neutrophils. NHL mostly shows monotonous population of premature lymphoid cells. For classifying both HL and NHL to different WHO subtypes, immunohistochemistry is very much essential. Immunohistochemistry also helps to assess prognosis and therapy.

Aims and Objectives

To evaluate the role of IHC in the typing and sub-typing of lymphomas according to WHO classification. Histomophological picture in H&E provided guidance for appropriate selection of immune panel for IHC.

Materials and Methods

Total 98 cases of histologically diagnosed lymphoma including HL and NHL cases (according to WHO classification) were reviewed from previous histopathology record in S.C.B Medical College, Cuttack, a premier institute in Odisha, India during Jan 2014 to Feb 2017, a period of 3years.These cases were routinely stained by H and E from formalin fixed paraffin embedded tissue. These cases were further subjected to immunohistochemical study after antigen retrieval, antibody labeling was done by streptavidin biotin peroxidase complex. IHC panel constituted of B cell and T cell markers like CD20, CD15, CD30, CD45, CD23, CD3, CD4, CD5 (table-4)[2], histiocyte marker CD68 were used. Epithelial cell markers like Pan-CK, CK-7, CK-20 and EMA were also done to differentiate lymphomas from metastatic or primary poorly differentiated carcinoma, undifferentiated carcinoma and round cell tumor in doubtful cases. Thus 6 cases with positive epithelial cell markers were excluded from our study and 92 cases were selected for IHC study for diagnosing the type of lymphomas as HL or NHL and also sub-typing different sub categories of HL and NHL according to WHO.

Results

Out of total 92 cases diagnosed as lymphoma, 13cases (14.2%) were HL and 79 cases(85.8%) were NHL, extranodal lymphoma constituted 28cases(35.4%) of all NHL. Total 13 cases of HL were studied. Most of the cases detected in young males. Male to Female ratio was 5.5:1. Maximum no of cases were seen below 10 years, youngest patient in this series was a 5years old. No case was found beyond 6th decade. Maximum number of cases i.e. 5 cases were found within the age group of 10years followed by 3 cases each in the age group of 31-40 years and 41-50years (table-1). The most common presenting clinical signs and symptoms were irregular fever with lymphadenopathy. Cervical lymphnodes were commonly involved in 11 cases, and in other two cases there was generalized lymphadenopathy along with hepatosplenomegaly.

Male to female ratio was 1.82:1 in cases of NHL. All age groups affected were ranging from 5-90 years of age. But maximum no. of cases were seen in the age group of 41-50 years (30.3%) (4) followed by 51-60 years(16.2%) (Table-2). Among B cell NHL cases, diffuse large B cell Lymphoma was the most common type (49%)(Table-3). In T-cell NHL, commonest type was lymphoblastic lymphoma followed by anaplastic large cell lymphoma. One case clinically and histologically diagnosed as HL came out to be anaplastic large cell lymphoma after IHC study.

Table-1 Types of Hodgkins Lymphoma

Sl no.	Type of HL	No. of cases	Age 0-10	11-20	21-30	31-40	41-50	>50
1.	Nodular sclerosis	7	2	1	nil	2	2	Nil
2.	Mixed cellularity	1					1	
3.	Lymphocyte rich	4	3	1				
4.	Lymphocyte depleted	Nil						
5.	Lymphocyte predominance	1					1	

Table-2 Age and sex distribution of NHL(n=51)

Sl no.	Age(in years)	Male	Female	Total	Percentage
1.	0-10	3	2	5	6.3%
2.	11-20	4	--	4	5%
3.	21-30	5	1	6	7.5%
4.	31-40	3	4	7	8.8%
5.	41-50	15	9	24	30.3%
6.	51-60	7	6	13	16.2%
7.	61-70	9	3	12	15%
8.	71-80	4	3	7	8.8%
9.	81-90	1	--	1	1.2%

Table- 3 Immunohistochemical profile of NHL(n=51)

	B cell	Number of cases	IHC Markers
1.	Diffuse large B cell lymphoma	26	CD20+, CD45+, CD5-ve, PanCK-ve
2.	Small lymphocytic lymphoma	3	CD20+, CD5+, CD23+
3.	Follicular lymphoma	4	CD5+, CD10+, CD20+ve
4.	Mantle cell lymphoma	3	CD5+, CD20+, CD10-ve
5.	T cell rich B cell lymphoma	3	CD3+, CD5+(In T-cell), CD20+, CD45+, CD68+
	T cell		
1.	Precursor T lymphpblastic lymphoma	6	CD3+, CD68+(in histiocytes)
2.	Anaplastic large cell lymphoma	5	CD3+, CD4+, CD5+, CD30+, CD15-ve,CD20-ve, CD68+
3.	T cell proymphocytic lymphoma	1	CD45+,CD3+

Table-4 Distribution & IHC of Extranodal lymphoma(n=28)

Sl no.	Site	No. of cases	B cell	T cell	IHC Markers
1.	G.I Tract	12	12	_	CD20+, CD45+, Pan-CK-ve
2.	Breast	2	1	1	CD20+, CD45+ CD3+(Tcell)
3.	Bone	3	2	1	Cd20+, CD45+, Pan-CK-ve, S100-, Cd31-, CD3+(Tcell)
4.	Pharyngeal	5	3	2	CD20+, Tcell- CD3+, CD20-, CD30-
5.	Tonsil	1	_	1	CD45+, Pan-CK-ve
6.	Oral cavity	1	1	_	CD20+
7.	Ovary	1	1	_	CD45+, Pan-CK-ve
8.	Parotid	1	1	_	CD20+, CD3-, Pan-CK-ve
9.	Retroperitoneal	1	1	_	CD20+, Pan-CK-ve
10.	Spleen (T cell rich B cell lymphoma)	1	1	_	CD20+

Discussion

Immunohistochemistry is an integral part of diagnostic histopathology. IHC with various antibodies help to determine the stage of development and the specific lineage of lymphoma cells. HL is classified into four subtypes based on histomorphological study using H&E stain (table-1). Hodgkins lymphomas are diagnosed histopathologically by identifying diagnostic RS cells in a background of reactive lymphocytes, histiocytes, plasma cells, eosinophils, neutrophils and fibroblasts. After the morphological diagnosis, all cases were subjected to immunohistochemistry using CD30, CD15, CD45, CD20, CD3 for confirmation and sub typing. As RS cell is considered the hallmark for the diagnosis of HL irrespective of subtype it was found to be positive for CD 15 and CD 30[7] except in mixed cellularity where RS cell exhibited CD 30 positive and CD 15 negative phenotype. In one case of lymphocyte predominance: CD 20 was positive, CD 3 and CD 30 were negative. In this series 7 cases (53.8%) were diagnosed as nodular sclerosis, 4 cases (30.7%) of lymphocyte rich and one case each of mixed cellularity and lymphocyte predominance(7.6%).

In this series, nodular sclerosis was found to be the most common variant that correlates with the other studies in our country[6]. Also CD30 and CD 15 were found to be positive in most of the cases.[7] So, in addition to the classification of HL immunohistochemistry is also helpful in arriving at the final diagnosis by excluding other diseases that has similar histomorphological picture. Out of all NHL cases, nodal involvement was seen in 51 cases and extranodal involvement is 28 cases and the ratio is 1.82:1. In this study, the extranodal involvement commonly seen because of large number of cases of maltomas and gastrointestinal lymphomas in comparison to other studies.[17,18] After immunohistochemical analysis it was found that, B cell NHL is the commonest type of NHL compared to T cell type i.e.76% B cell origin and 24% T cell origin. These findings are similar to other studies.[6,9]

Among B cell NHL, commonest type is DLBCL with highest percentage. 26 cases of DLBCL accounting for 50.9% comparable to other studies.[5,8,10] Follicular lymphoma- 4 cases (9%) and SLL/CLL- 3 cases (6%) is lower in incidence in comparison to other countries.[9,13,14] Out of T cell NHL, T cell acute lymphoblastic- 6 cases (12%).[9,12] ; this is compared to studies in other part of India.[9,12] ALCL- 5 cases (10%) of all NHL cases. In our series one case of 4 years old male child histologically diagnosed as metastatic neuroblasoma after IHC study: NSE-ve, chromogranin –ve but CD45+ve & CD3+ve, so diagnosed as NHL.

Conclusion

Immunophenotyping is required for the diagnosis and classification of malignant neoplasms of lymphoid origin. Even if the diagnosis is done basing on histomorphology, to avoid any diagnostic pitfall, interpretation of IHC marker studies is mandatory. IHC study is also helpful in predicting the prognosis and guidance of appropriate therapy.

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