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Original Research Article

Role of Immunohistochemistry and Its Correlation with Histopathological Diagnosis of Lymphomas

Narmatha M.[1], Maheswari J.[2], Premalatha A.[3]

[1,2,3]Assistant Professors, Department of Pathology, K.A.P. Viswanatham Government Medical College, Tiruchirappalli, Tamilnadu, India

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Corresponding author: Dr Maheswari J.
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Abstract

Introduction: Currently, the diagnosis of lymphomas relies on immunophenotyping in addition to morphological features and relevant clinical data. According to WHO classification, lymphomas are categorized into Hodgkin's and Non-Hodgkin's lymphomas, further subclassified as T cell, B cell and NK cell types based on the cell lineage, stage of maturation and function. Appropriate selection of panel of antibody markers according to histopathological diagnosis is essential for definite and accurate diagnosis.

Aims and Objectives: (1) To analyze the incidence and spectrum of morphological features of lymphomas. (2) Role of immunohistochemistry in subtyping of nodal and extranodal lymphomas and its correlation with histopathological diagnosis.

Materials and Methods: This retrospective study includes 30 cases received in the Department of Pathology, KAP Viswanatham Govt Medical College, Trichy from January 2019 to December 2021. The distribution of cases were determined according to age, sex and site. Formalin–fixed, paraffin-embedded tissue sections were stained with Hematoxylin and eosin stain., Histopathological diagnosis based on revised 2016 WHO classification and final diagnosis using panel of IHC markers was done.

Results: Among 30 cases, 18 cases(60%) were females and 12 cases (40%) were males. Female to male ratio 3:2. The predominant age group being 50-60 years followed by 40-50 years. Non-Hodgkin's lymphoma (80%) were found to be more common than Hodgkin's lymphoma(20%). In NHL, B-cell lymphomas accounted for 80% and T-cell lymphomas 20% among which most common subtype is Diffuse large B cell type (37.5%), followed by Follicular lymphoma (25%), Peripheral T cell lymphoma (16%), Marginal zone lymphoma (0.5%), Lymphoblastic lymphoma (0.5%) and NK/T cell lymphoma (0.5%).

Conclusions: NHL is more common than HL. To avoid pitfalls in the diagnosis of lymphoma , clinical details, definite pathological criteria for each lymphoma and its correlation with immunophenotyping, molecular genetic testing, and cytogenetical analysis are essential.

Keywords: Non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma (HL) and Immunohistochemistry (IHC).

Introduction

Based on the current WHO classification, lymphomas are broadly divided into two major categories - Hodgkin's lymphoma and Non-Hodgkin's lymphoma. NHL is further subclassified based on determination of cell lineage, maturation and function, each with specific morphologic and immunophenotypic patterns as, B-cell, T-cell, and NK-cell type [1]. The diagnosis of HL is based on the presence of Reed-Sternberg (RS) cells along with varying proportions of eosinophils, neutrophils, epitheloid histiocytes, fibroblasts and immunophenotype of RS cells.

Recently immunohistochemistry has become an essential diagnostic tool. In the present scenario, combined interpretation of histopathological findings together with immunohistochemistry has become a strong basis for diagnostic, targeted therapeutic and prognostic implications [2].

Since no single marker is specific and to avoid pitfalls in the interpretation of immunohistochemical analysis, appropriate selection of antibody panel based on morphology is essential for achieving accurate diagnosis [3].

Material and Methods

This study was conducted at KAP Viswanatham Govt Medical College & Hospital, Trichy, Tamilnadu, from January 2019 to December 2021 and includes totally 30 cases of lymphoma. As a part of this retrospective study, both small and completely resected nodal and extranodal specimens were received. Clinical data including age,sex,site,and relevant investigation details were also obtained. Gross examination was done, of which size, shape, consistency and appearance were noted. Extensive bit taking was carried out, after which formalin fixed paraffin embedded tissue sections were made and stained with routine Hematoxylin and eosin stain.

The panel of monoclonal antibodies used for immunohistochemistry included CD15, CD20, CD45, CD3, CD30, Cyclin D1, Bcl2, Bcl6, CD56, CD10 and CD99. Antibodies against cytokeratin, epithelial membrane antigen (EMA),S-100 and synaptophysin were used in specific cases to rule out epithelial carcinoma, melanoma and neuroendocrine tumor. Final diagnosis was made and all lymphomas were classified according to the revised WHO classification (2016).

Observation and Results

The present study shows NHL (80%) are more common compared to HL (20%) as depicted in Table- 1.

Table 1: Incidence of Hl & Nhl

S.No	Diagnosis	No of Cases	Percentage
1	HL	6	20%
2	NHL	24	80%
	Total	30	100%

Table 2: Age Wise Distribution

S.No	Age in years	No of cases
1	< 10 yrs	0
2	11-20	2
3	21-30	3

4	31-40	4
5	41-50	7
6	51-60	10
7	61-70	4

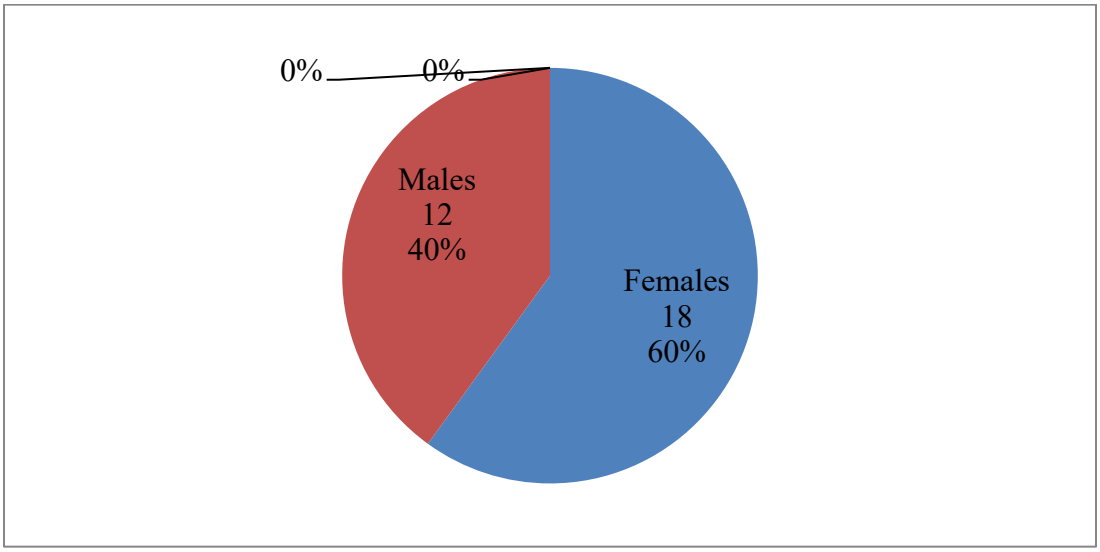

Chart 1: Sexwise distribution

Table 3: Site Wise Distribution Of Hl &Nhl

Nodal lymphomas			
S.no	Site	HL	NHL
1	Cervical	3	7
2	Axillary	1	6
3	Inguinal	-	1
4	Submandibular	1	1
5	Submental	1	-
	Total	6	15
Extranodal lymphomas			
1	Soft tissue	-	1
2	Lung	-	1
3	Stomach	-	1
4	Small intestine	-	4
5	Large intestine	-	1
	Total	-	9

Of 24 cases of NHL 15 cases (50%) were nodal and the remaining 9 cases (30%) were extranodal. Cervical nodes are the most common site followed by axillary nodes and submandibular nodes. Also, the most common extranodal site is GIT followed by soft tissue. All 6 cases of HL were found to be nodal origin and there is no extranodal HL cases in our study as shown in Table 3.

Among the 6 cases of HL, Lymphocyte-rich variant (50%) was the most common subtype followed by Mixed cellularity (36.4%) and Nodular sclerosis (16.6%) as shown in Table 4.

Table 4: Distribution of Sub Types of Hl

S.no	HL TYPE	NO of cases	Percentage
1	Lymphocyte rich	3	50%
2	Mixed cellularity	2	33.4%
3	Nodular sclerosis	1	16.6%
	Total	6	100%

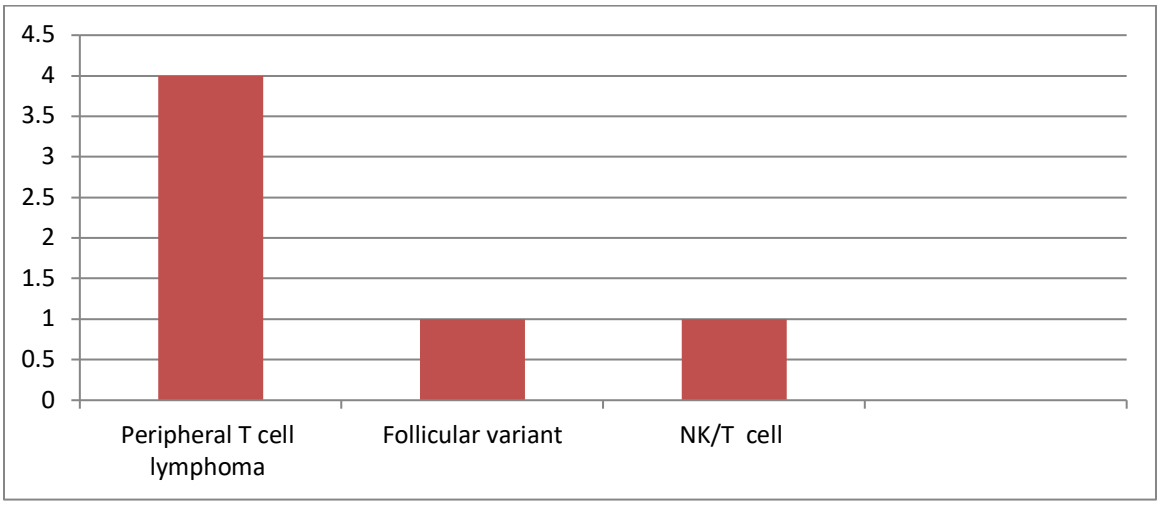

Chart 2: Distribution of NHL T cell type

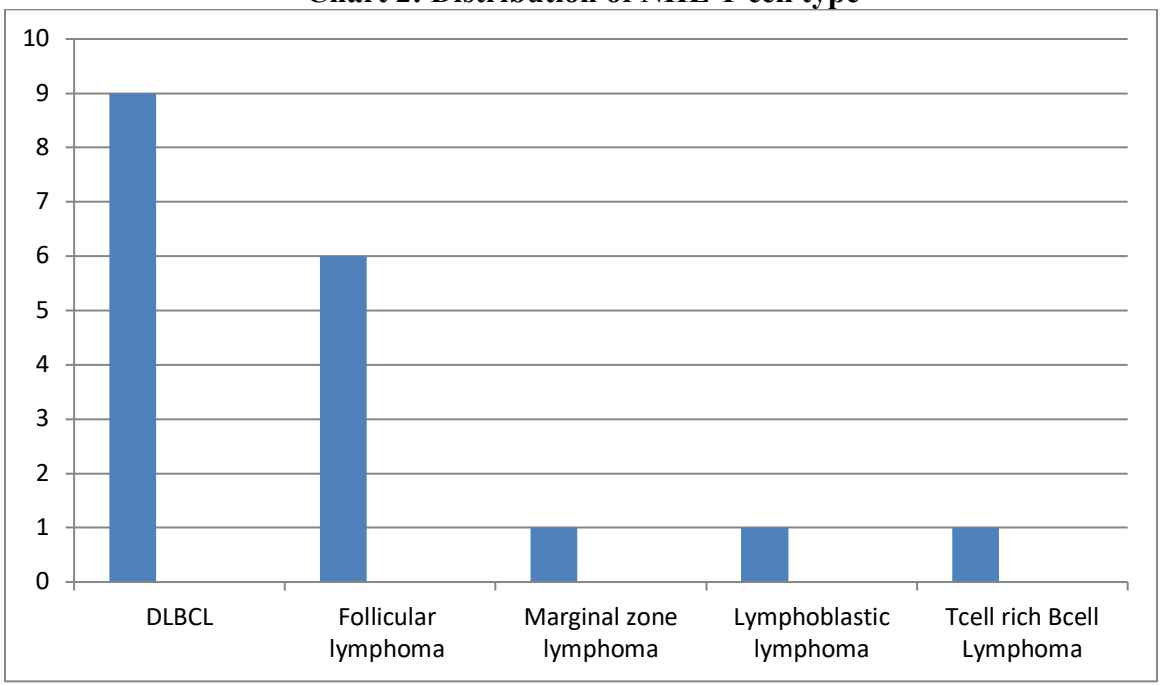

Chart 3: Distribution of NHL B cell type

Table 5: IHC Panel

	Lymphomas	CD3	CD5	CD10	CD15	CD20	CD30	CD45	CD56	CD99	BC12	Bcl6	cyD1	Ki67
1	DLBCL					++	+/-	++						++
2	Follicular	Focal +ve	-	++		++		+			++	++		
3	FL – CDS variant		++	+		++	-	+			+	-		++
4	T Cell rich Bcell	+	-	-	-	++		+			Variable	Variable		
5	Marginal Zone	-	-	-		++		-			++	-		
6	Lympho blastic	+		++		Variable		-		++	-	-		
7	Peripheral T Cell	++	+			Variable		+						
8	Nk/T cell	++	+			Weak	Variable	Variable	++					++
9	Hodgkins				++	+/-	++	++						

There were 3 cases where histopathological diagnosis does not correlate with IHC diagnosis. Among these, 2 cases of poorly differentiated carcinoma on HPE which on IHC showed.

CD20 positive, EMA and CK negative, confirming the diagnosis of NHL - B cell lymphomas. Similarly, one case of neuroendocrine carcinoma on HPE showed diffuse CD20 positivity with Synaptophysin and S-100 negativity. The present study shows 90-95% of HPE correlation with immunohistochemistry.

Discussion

The present study says that IHC is the final diagnostic tool for confirmation as well as subtyping of lymphomas. Histopathological diagnosis is the initial step followed by IHC. Histopathological examination included architectural alterations (partial or complete effacement), region of lymph node involved (follicular centre, mantle, marginal, Interfollicular. or sinus area), population of abnormal cells (monomorphic or polymorphic), pattern (nodular or diffuse), cell size (small, intermediate or large) and nuclear features (round, irregular, cleaved, with condensed or dispersed chromatin, presence or absence of nucleoli).

Based on morphology recommended basic IHC panel included antibodies against T cell antigens (CD3, CD5), B Cell antigens (CD20), and other antibodies like Bcl2, Bcl6, CD15, CD30 and CD45 would be helpful to further categorize the process.

Also EMA, CK, S100 and synaptophysin are used in specific cases to rule out epithelial carcinomas, melanomas, and neuroendocrine tumors.

The present study shows B cell lymphomas (80%) surpassed T cell lymphomas (20%) similar to the incidence and distribution as shown by Mustaq S et al [4] and Sharma et al [5] in their respective studies (Table-6).

Table 6: Incidence of B cell and T cell lymphomas in various studies

Histopathological Diagnosis	Present study	Mustaq S et al.(2005)	Sharma M et al.(2014)
B cell lymphomas	80%	86%	89.3%
T cell lymphomas	20%	24%	10.7%

In our study, approximately 70% are nodal and remaining 30% are of extranodal origin. The median age of study population was found to be 50-60 years which is similar to a study conducted by Vallabhajosyula et al 2010 [6].

Among NHL most common subtype is Diffuse large B cell type (DLBCL-37.5%),followed by Follicular lymphoma(25%), Peripheral T cell lymphoma(16%), Marginal zone lymphoma(0.5%),Lymphoblastic lymphoma(0.5%) and NK/T cell lymphoma(0.5%) in our study(Table 7).

Table 7: Distribution of B cell lymphoma subtypes in various studies

Category	Present study	Padhi S et al. [10] (2012)	Howell et al. (2012) [11]	Sharma M et al. [5] (2014)	Rao A et al. [12] (2013)
DLBCL	37.5%	69%	47%	46.8%	29.3%
FL	25%	8%	8%	12.8%	6.8%
Marginal zone lymphoma	4.3%	3%	5%	3%	4%

DLBCL accounts for 36-38% of NHL and is diagnosed by the presence of diffuse pattern of large transformed lymphocytes with prominent nucleoli. Immunostaining shows strong expression with CD3, CD20, CD45, weak CD30, and high proliferation rate of Ki-67. T cell rich B cell lymphoma is a special subtype of DLBCL and is identified by haphazardly scattered single cells or tiny clusters of neoplastic B cells in a background of T cells expressing CD20, CD3, CD45 positivity with variable expression of Bcl2 and Bcl6.

Next most common subtype of NHL is follicular lymphoma which characterized by uniform sized neoplastic follicles composed of small to intermediate sized lymphocytes along with centrocytes, centroblasts and infrequent mitoses. Grading is based on the proportion of centroblasts/hpf. CD10 is highly expressed followed by Bcl2, Bcl6 and pan B cell markers. Grade 3 follicular lymphoma (FL) usually lacks CD5 expression but FL CD5 variant shows more than 15% centroblasts with increased mitosis and > 20% Ki 67 [7].

In the present study, one of 6 cases of FL (4%) at our institution were found to be CD5 positive of grade 3 morphology with high mitotic index. Very few cases have been described in the literature [8]. Though this variant is very rare, it needs to be considered in the differential diagnosis with other CD 5 positive B cell lymphomas including DLBCL, MALToma, Splenic marginal zone lymphoma and Lymphoplasmacytic lymphoma. Miyoshi et al in Japan reported in his study that pathology with CD5 positive Follicular Lymphoma showed a higher frequency of peripheral blood involvement and transformation to DLBCL with poor prognosis [9].

Lymphoblastic lymphoma is identified by effacement of nodal architecture by nodular or diffuse infiltration of interfollicular areas by small lymphocytes, atypical blastoid cells and plasma cells expressing diffuse positivity for Pan B-cell markers CD3, CD10, CD99, variable for CD45, CD20 and negative for

cyclin D1. CD99 positivity distinguishes Lymphoblastic variant from Burkitt's lymphoma which is CD10 positive but CD99 negative.

Marginal zone lymphoma showed varying sized follicles with prominent onion skin mantle zone, widened paracortex with numerous vessels containing small lymphocytes, histocytes and patchy neutrophils. CD20 and Bcl2 were strongly positive whereas CD3, CD5, CD10, Bcl6 and cyclin D1 were found to be negative. (Table 4)

Though T cell / NK cell neoplasms were common and difficult to diagnose, they should be considered at extranodal sites such as skin, nose and GIT due to angioinvasion and zonal necrosis They have no definite morphological features and mostly have a reactive appearance with a mixture of small and large lymphocytes in the inflammatory background. For T cell lymphomas CD2, CD3, CD4, CD5, CD7, CD8 and CD30 will be useful which shows membranous staining. The diagnosis of NK cell neoplasm is made out after T cell lineage is excluded. NK cells lacks CD5 and express NK associated antigens CD56 or CD57.

HL accounting for 15% of lymphomas are characterised by low number of B cell derived pathognomonic, malignant Reed-Sternberg (RS) cells in an extensive inflammatory microenvironment. Histopathologically, HL are classified as classical (cHL) and Nodular lymphocyte predominant (NLPHL) based on morphology and IHC. cHL further subdivided into four sub-types, based on morphology, abundance of RS cells and the background infiltrate. Nodular sclerosis is the most common cHL(70%) characterised by neoplastic lacunar RS cells in an inflammatory background of band-forming sclerosis. Mixed-cellularity subtype

shows RS cells scattered in a diffuse mixed inflammatory background without sclerosis. Lymphocyte rich cHL have scattered RS cells within nodular or diffuse cellular background of small lymphocytes and without neutrophils or eosinophils. Lymphocyte depleted is the rarest and most aggressive cHL with diffuse infiltration by RS cells without a significant reactive inflammatory infiltrate. NLPHL is characterised by LP cells in a background of small lymphocytes admixed with histiocytes. LP cells have enlarged nuclei with a lobular contours and prominent nucleoli. The characteristic immunophenotyping of RS cells in cHL is CD30 expression positivity virtually in all cases followed by CD15. NLPHL is, CD45, CD20 and PAX 5, OCT2 and Bcl6 positive, CD30 variably positive, CD15 mostly negative, and CD10 negative. NHL is confirmed by lack of expression of CD45, CD20 and PAX 5 differentiating it from NLPHL.

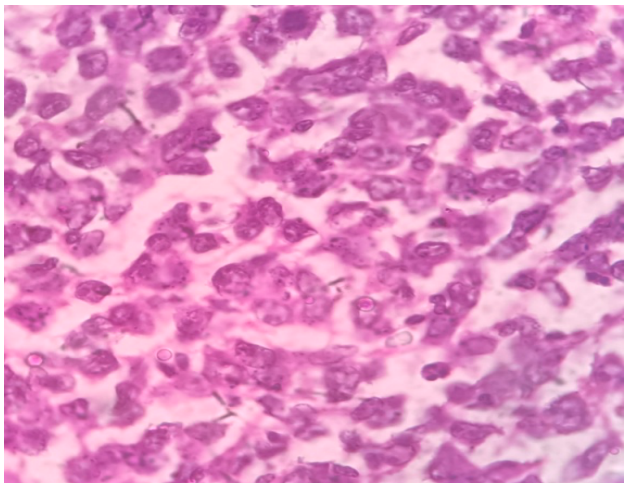

Figure 1: H & E of DLBCL (40X)

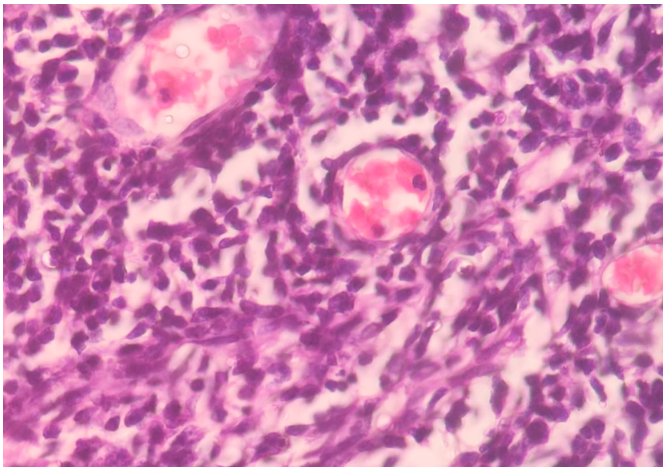

Figure 2: H & E of NK Cell Lymphoma (40X)

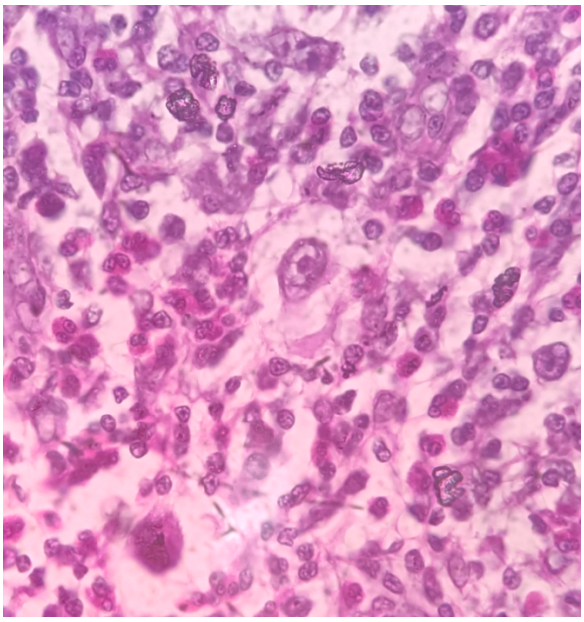

Figure 3: H & E of Hodgkins Lymphoma (40X)

IHC Images

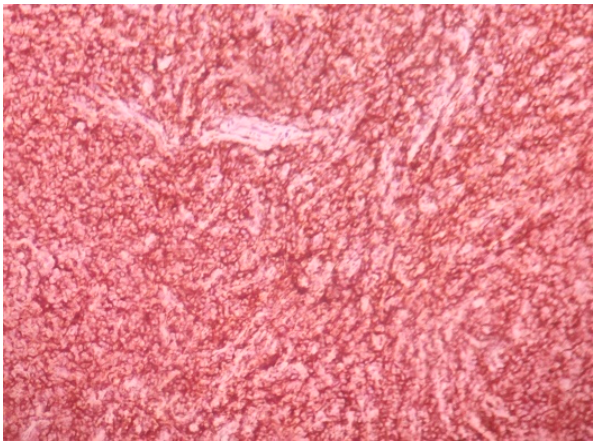

Figure 4: IHC - CD20 Positive for DLBCL

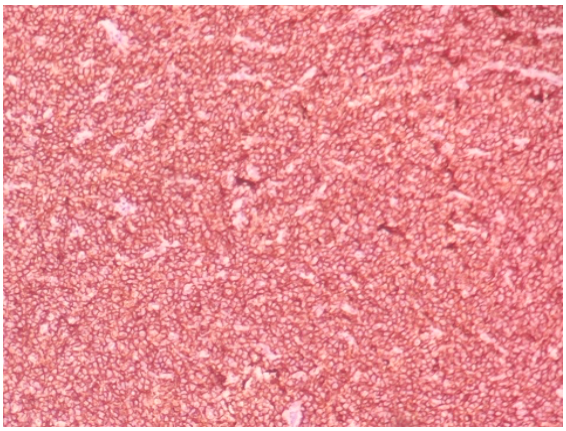

Figure 5: IHC - BCL2 Positive for Follicular Lymphoma

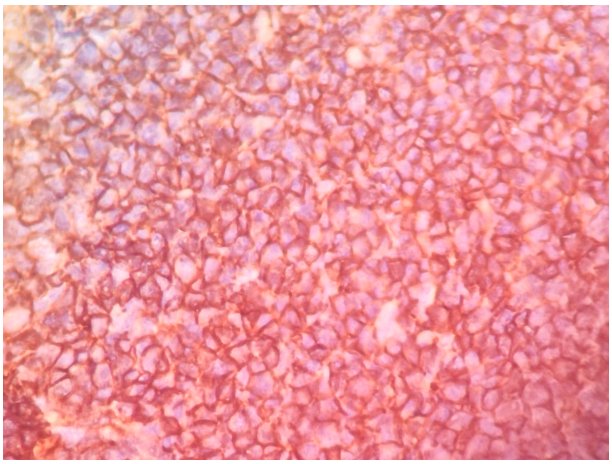

Figure 6: IHC - CD99 Positive for Lymphoblastic Lymphoma

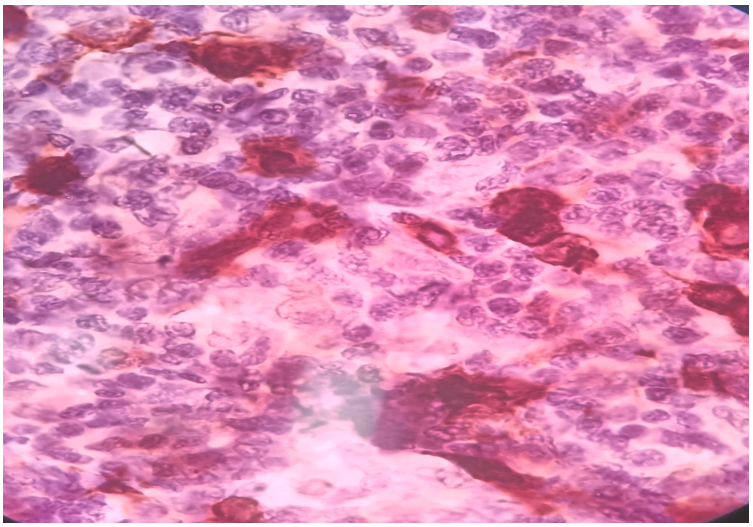

Figure 7: IHC – CD30 Positive for Hodgkins Lymphoma

Conclusion

Immunohistochemistry plays an important role in confirmation, typing and subtyping of lymphomas. Each lymphoid neoplasm has a characteristic morphological and immunophenotypically features. Since no single antigen is lineage specific, and to avoid potential pitfalls in the diagnosis, immunostaining must be performed in the context of a panel. Also, familiarity with the definite histopathological criteria, differential diagnosis of each lymphoid tumor, concise immunophenotypic panel, its correlation with pathological findings, ancillary molecular cytogenetic studies and relevant clinical history are mandatory for achieving diagnostic, prognostic and therapeutic implications of nodal and extranodal lymphomas.

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