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Histomorphology of Lymphomas

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ABSTRACT

Context: *Diagnosis of lymphoma constitutes a difficult task in context to subtyping. Accurate diagnosis is essential because the treatment options, responses to therapy and prognosis vary widely depending on the diagnosis. However to make a definitive diagnosis of a lymphoma based solely on the H & E light microscopy, findings may be exceedingly difficult because of frequent absence of distinguishing features. IHC represents a tool that can provide a clear distinction among the different types of lymphoma. The purpose of IHC is to categorize the patient in order to ensure appropriate and specific treatment, as well as to identify tumours at higher risk of recurrence and fatal outcomes.*

Aims: *To study histomorphology of different lymphomas and correlation of it with immunohistochemical findings.*

Settings and Design: *The present study is a retrospective as well as prospective study of lymphoma cases diagnosed based on routine histopathology as well as correlation of it with immunohistochemistry in surgical pathology section of the Central Diagnostic Laboratory, Shree Krishna Hospital, Karamsad from September 2010 to August 2015.*

Materials and Methods: *A detail clinical history, nature of specimen and other investigations were noted in the proforma. Macroscopic and microscopic findings or any incidental findings were documented in the final report.*

Results: *Total 47 cases of lymphoma were diagnosed in study period among them 42 cases were of B cell lymphoma and 7 cases were of T cell lymphoma. All B cell lymphoma were positive immunihistochemically for CD 20 and LCA. Out of 47 cases 25(53.19%) were of lymphoid origin and 22(46.81%) were of extralymphoid site.*

Conclusion: *From this study it was concluded that:*

- *In all the cases diagnosis of hematoxyllin and eosin stained sections were correlated with immunohistochemical findings*
- *Non Hodgkin's Lymphoma were most common than Hodgkin's lymphoma*
- *Lymphnodes are the most common site of origin.*

Introduction

World Health Organization broadly classifies lymphomas into Hodgkins lymphoma (HL) and non-Hodgkins lymphoma (NHL). Non-Hodgkins lymphoma is further sub classified based on the stage of maturation (immature vs. mature) and cell of origin [B cell, T cell, or natural killer cell (NK) cell].[1]

Morphologic assessment takes into account the anatomic architectural alterations in the lymphoid compartment [i.e., B-cell follicle (follicle center, mantle, or marginal zone) or T-cell regions (interfollicular or sinus areas)]. If an abnormal population is present (polymorphic or monomorphic), the determination of pattern (diffuse or nodular) and cell size (small, intermediate, large) and nuclear characteristics (round, irregular, cleaved with condensed or dispersed or blastic chromatin, and the character of the nucleoli) is made.[1]

A 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 cytoarchitectural pattern). [2,3]

This study was carried out to study histomorph-ology of different lymphoma and correlation of it with immunohistochemical findings.

Aims and Objectives

1. To study histomorphology of different lymphomas.
2. Correlation of H & E findings with immunohistochemistry findings.

Materials and Methods

The present study is a retrospective as well as prospective study of cases surgical biopsy or specimen of lymphnodes received for histopathological study at the surgical pathology section of the Central Diagnostic Laboratory, Shree Krishna hospital, Karamsad from September 2010 to August 2015.

Tissue from patients of all age groups, who were diagnosed on routine histopathological examination as well as on immunohistochemistry as lymphoma were taken as sample for study .

A detail clinical history was noted in the proforma. Specimens were received in 10% formalin, following which specimen identification, patient identification, type of surgery and organ identification were done. Detailed gross examination was recorded under heads of overall appearance, size and appearance of cut surface. Additional cuts were made depending on the size of the specimen.

Specimens were fixed as early as possible by 10% neutral buffered formalin and processed preferably within 24 hours of surgery of patient. Tissue bits from the representative area were taken for histopathological examination. After routine paraffin processing, sections were cut 5-6 µm thick for hematoxylin-eosin stain. The blocks were sectioned and stained with haematoxylin and eosin stain.

Macroscopic and microscopic findings or any incidental findings were documented in the final report.

After histopathological examination, the slides and blocks were sent at Baroda Clinical Laboratory, Vadodara or SRL Diagnostic Laboratory, Mumbai as per patient convenience for immunohistochemical study.

Further, the obtained parameters were evaluated using descriptive statistical analysis. Statistical analyses were performed manually as well as using the Microsoft Excel 2013.

Inclusion criteria: Tissue from patients of all age group which were diagnosed on routine histopathological examination as lymphomas and confirmed with immunohistochemistry were taken as sample for study. Specimens other than Shree Krishna Hospitals were also taken as sample in the study.

Exclusion criteria: The cases which were suggestive of lymphoma on routine

histopathological examination but not confirmed on immunohistochemical study were excluded from the study.

Observations and Results

The present study was undertaken to study the correlation of histomorphology and immuno-histochemistry for lymphomas and for further subtyping of the different types of lymphoma in the Department of Pathology, Pramukh Swami Medical College, Karamsad.

Total 47 cases reported as lymphoma, using routine haematoxylin and eosin stains as well as immunohistochemistry; were included in my study.

The histopathology and immunohistochemistry reports of 47 tumours were reviewed and assigned to appropriate category.

A basic panel of antibodies were applied first with respect to the patient‘s age, tumour location and cell pattern and then additional antibodies were used for final diagnosis.

However applications of all markers were not done in all cases due to technical problem/ block exhaustion/ non-availability of antibodies at that time. Result of the present study were analyzed in the following tables.

Table:1 Distribution of lymphoma cases

Site	No of cases	Percentage
Nodal	25	53.19%
Extranodal	22	46.81%
Total	47	100.00%

In present study out of 47 cases, 25(53.19%) cases were in lymphoid tissue and 22(46.81%) cases were in extranodal site.

Table: 2 Distribution of lymphoma cases according to site

Organ/tissue	Number of cases	Percentage
lymphnode	25	53.19%
brain	4	8.51%
nose	3	6.38%
liver	3	6.38%
breast	2	4.26%
Terminal ileum	2	4.26%
stomach	2	4.26%
omentum	1	2.13%
caecum	1	2.13%
rectum	1	2.13%
tonsil	1	2.13%
umbilicus	1	2.13%
lung	1	2.13%

Out of 47(58.02%) cases of lymphoma, the most common site was lymph node in 25(53.19%) cases followed by brain in 4(8.51%) cases. As seen from the table 6, the lymphoma was distributed among other sites.

Table 3: Immunohistochemical study in Lymphoma

subtype	No.	LCA	CD20	CD3	CD5	CD10	CD23	CyC D1	bcl-2
DLBCL	23	23 (100%)	23 (100%)	0 (0%)	0 (0%)	7 (30%)	0 (0%)	0 (0%)	0 (0%)
Follicular lymphoma	9	9 (100%)	9 (100%)	0 (0%)	0 (0%)	6 (66%)	1 (11%)	0 (0%)	9 (100%)
Mantle cell lymphoma	2	2 (100%)	2 (100%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)
Marginal Zone lymphoma	3	3 (100%)	3 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
SLL/CLL	3	3 (100%)	3 (100%)	0 (0%)	3 (100%)	0 (0%)	3 (100%)	0 (0%)	0 (0%)
B cell NHL	2	2 (100%)	2 (100%)	0 (0%)	1 (50%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)
T cell NHL	3	3 (100%)	0 (0%)	3 (100%)	1 (33%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
T cell ALL	2	2 (100%)	0 (0%)	2 (100%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)

Only 5(10.63%) cases were of T cell type of which 3 cases (6.38%) were of T cell lymphoblastic lymphoma and 2 cases (4.25%) were of T cell NHL.

The remainder 42(89.36%) cases were of B cell lymphoma which includes 23(54.76%) cases of diffuse large B cell lymphoma, 9(21.42%) cases of follicular cell lymphoma, 3(7.14%) cases each of marginal and small lymphocytic lymphoma/leukemia and 2(4.76%) cases each of mantle cell lymphoma and B cell lymphoma.

LCA and CD20 were positive in 42(100%) cases which includes 23(100%) cases of diffuse large B cell lymphoma, 9(100%) cases of follicular cell lymphoma, 3(100%) cases of marginal cell lymphoma, 3(100%) cases of small lymphocytic lymphoma/leukemia, and 2 (100%) cases of mantle cell lymphoma and 2(100%) cases of B cell lymphoma.

CD 3 was positive in 5(11.9%) cases of Non-Hodgkin's Lymphoma T cell type which include 3(100%) cases of T cell NHL and 2 cases (100%) of T cell ALL.

CD 5 was positive in 7(16.66%) cases of Non-Hodgkin's Lymphoma T cell type which includes 2(100%) cases of mantle cell lymphoma, 3(100%) cases of SLL/CLL, 1(50%) case of B cell lymphoma and 1(33.33%) case of T cell NHL.

CD 10 was positive in 14(33.33%) cases which includes 7(30.43%) cases of diffuse large cell lymphoma, 6(66.67%) cases of follicular lymphoma and 1(50%) case of T cell ALL.CD 23 was positive in 5(11.90%) cases which includes 1(11.11%) case of follicular lymphoma 3(100%) cases of SLL/CLL and 1(50%) case of B cell lymphoma. Cyclin D1 was positive in 2(100%) cases of mantle cell lymphoma. Bcl-2 was positive in 9(100%) cases of follicular lymphoma. Bcl-6 was positive in 14(33.33%) cases which includes 5(21.73%) cases of diffuse large B cell lymphoma and 9(100%) cases of follicular lymphoma.

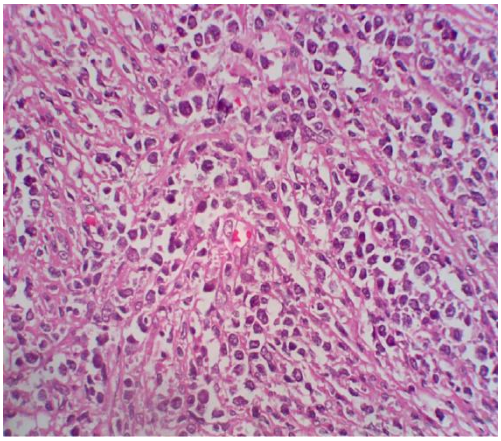

Fig-1 H & E (x 400) - Diffuse Large B cell lymphoma of cervical lymph node

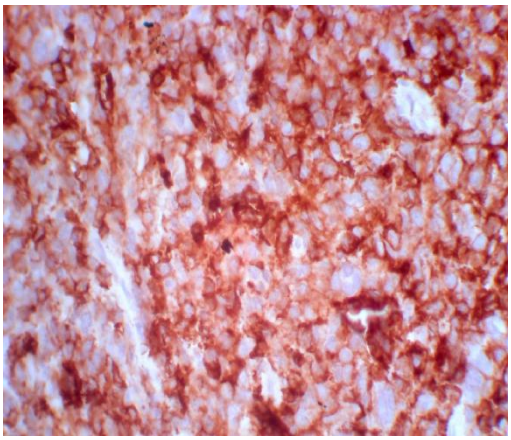

Fig-2 Immunohistochemistry (x 400) LCA (CD45)

Discussion

In present study, lymph node was the most common site which was involved by the tumour. The study done by Patel MM et al.[4] found lymph node as most common site. Bashyal R et al.[5] found nasopharynx as most common site of involvement. This can be explained by exclusion of cases of nodal Non-Hodgkin's lymphoma from his study.

Site wise distribution of Lymphoma:

Out of 47 cases, 25(53.19%) cases were in lymphoid tissue and 22(46.81%) cases were in extra nodal site. The studies conducted by Das et al.[6] Frank D et al.[7], Glass et al.[5] and Patel MM et al.[4] found similar results.

Table 4 comparison of distribution of lymphoma cases according to site in various studies.

Study	Nodal	Extra nodal
Das et al [6](1994)	83.33%	16.67%
Glass et al [5] (1997)	71.90%	28.10%
Frank D et al [7] (2000)	72.72%	27.28%
Patel MM et al [4] (2013)	57.15%	42.85%
Present study	53.19%	46.81%

An interesting findings of these data were that, in recent years relatively large number of cases were designated as extra nodal. These may be due to improvement in the diagnostic facility as well as awareness of patients regarding diagnostic modalities as compared to past.

Age wise distribution of Lymphoma:

In present study, maximum number of lymphoma cases were present in 61-70 yr. age group. The study conducted by Patel MM et al.[4] also showed similar results.

Table 5 shows comparison of age wise distribution of lymphoma cases.

Table 5 Comparison of various studies according to the age wise distribution of Non-Hodgkin's Lymphoma cases

Study	61-70 yrs. age group	71-80 yrs. age group
Glass et al.[5] (1997)	23.7%	24.5%
MM Patel et al.[4](2013)	13.75%	3.75%
Present study	24.69%	12.35%

Sex wise distribution of Lymphoma

In present study, males outnumbered females as 76.6% cases were of male patients and 23.4% cases were of female patients. The studies of D'cruze L et al.[8], Glass et al.[5], Padhi et al.[9], Mustaq et al.[10], Aparna et al.[11] and Sharma et al.[12] also concluded the same results.

Table 19 shows comparison of sex wise distribution of lymphoma cases in various studies.

Distribution of lymphoma cases:

Shah SH et al.[13] (1999) studied immunohistochemical evaluation of 9 (16.10%) cases of Non-Hodgkin's lymphoma. In the present study we studied [14] (58.02%) cases of Non-Hodgkin's lymphoma.

Konrad P et al.[15] (2009) found 1 (0.76%) case to be CD45 and CD20 positive. Further markers were applied and it was diagnosed as diffuse large B cell lymphoma. [16]

Bashyal R et al.[17](2011) studied 40 cases of small round cell tumours. Tumours presented at various sites. On histopathological examination, in 21(52.5%) cases had differential diagnosis of Non-Hodgkin's lymphoma and poorly differentiated carcinoma were given. All the 21(52.5%) cases showed CD45 positivity and were diagnosed as Non-Hodgkin's lymphoma.

D'cruze L et al. [18](2013) studied 43 cases of small round cell tumours presented at various sites. On histopathological examination 19(44.2%) cases had varied differential diagnosis like Non-Hodgkin's lymphoma, poorly differentiated carcinoma, Ewing's sarcoma and Rhabdomyosarcoma. All 19(44.2%) cases showed CD 45 positivity and were diagnosed as Non-Hodgkin's lymphoma. Out of 19(44.2%) cases, CD 20 was positive in 12(63.16%) cases which were diagnosed as Non-Hodgkin's lymphoma B-cell phenotype and CD3 was positive in 7(36.84%) cases which were diagnosed as Non-Hodgkin's lymphoma T cell phenotype. Similarly, in the present study, on histopathological examination 47(58.02%) cases had varied differential diagnosis like Non-Hodgkin's lymphoma and poorly differentiated carcinoma. On IHC study, in 42(51.85%) cases the tumour cells were positive for CD45 and CD20. They were diagnosed as Non-Hodgkin's lymphoma, B-cell phenotype. Negativity for CK excluded other undifferentiated carcinoma, whenever required. However, IHC study of 5(6.17%) cases were positive for CD5 which were diagnosed as Non-Hodgkin's lymphoma-T cell phenotype.

Patel MM et al.[4] (2013) studied 80 cases of small round cell tumours. Out of 80 cases, 26 cases (32.50%) were diagnosed as Non-Hodgkin's lymphoma on the basis of CD45 positivity. Out of 26 cases (32.50%), CD20 was positive in 21 cases (81.77%) which were diagnosed as Non-Hodgkin's lymphoma B-cell phenotype and CD3

was positive in 4(15.38%) cases which were diagnosed as Non-Hodgkin's lymphoma T cell phenotype. In 1(3.85%) case markers for B and T cell were equivocal. In the present study, 42(51.85%) cases were CD45 and CD20 positive which were diagnosed as Non-Hodgkin's lymphoma, B cell phenotype while 5 (6.17%) cases were positive for CD5 which were diagnosed as Non-Hodgkin's lymphoma, T cell phenotype.

Further subtyping of Non-Hodgkin's Lymphoma B cell phenotype was performed. Out of 26 cases, 18(57.14%) cases were of diffuse large B cell lymphoma, 3(7.14%) [20] cases were of Follicular B cell neoplasm, 1(3.57%) case each was of SLL/CLL, Mantle cell lymphoma, Lymphoplasmacytic lymphoma and Lymphoblastic lymphoma. In present study, out of 47 cases, 28(59.57%) cases were of diffuse large B cell lymphoma, 9(19.14%) cases were of Follicular B cell neoplasm, 3(6.38%) cases were of SLL/CLL, 2(4.25%) cases were of Mantle cell lymphoma, 3(6.38%) cases were of marginal B cell lymphoma and 2(4.25%) cases were of B cell neoplasm in which further typing could not be done due to lack of tissue in cell block.

Sharma et al.[12] (2014) studied 47 cases of Non-Hodgkin's lymphoma on the basis of CD45 positivity. Out of 47 cases, CD20 was positive in 42(89.36%) cases which were diagnosed as Non-Hodgkin's lymphoma B-cell phenotype and 5(10.64%) cases were CD3 positive which were diagnosed as T-cell Non-Hodgkin's lymphoma.

Further subtyping of Non-Hodgkin's Lymphoma B cell phenotype was done. Out of 42 cases, 22(46.8%) cases were of diffuse large B cell lymphoma, 8(17%) cases were of SLL/CLL, 6(12.8%) cases were of Mantle cell lymphoma, 3(6.4%) cases were of follicular lymphoma, 1(2.1%) case was of precursor B cell lymphoma, 1(2.1%) case was of diffuse mixed small cleaved cell and large cell type and 1(2.1%) case was of Marginal zone B cell lymphoma.

Further subtyping of Non-Hodgkin's Lymphoma T cell phenotype was done. Out of 5 cases, 3(6.4%) cases were of precursor T cell lymphoblastic lymphoma and 2(4.2%) cases were of mature T cell lymphoma. In present study, out of 5(4.2%) cases, 2 (4.2%) cases were of T cell ALL and 3(6.4%) cases were of T-cell NHL.

Subtype of lymphoma

In present study, diffuse large B cell lymphoma (DLBCL) was the most common subtype in Non-Hodgkin's lymphoma. In present study 48.93% of all NHLs were found to be DLBCL. In three different studies reported from Naresh et al.[22], Sharma et al.[23] and Sengar M et al.[24], incidence rate of DLBCL was found to be 34%, 46.8% and 42% respectively. Various studies reported from Roy et al.[25], Aparna et al.[26] and Kalyan et al.[21] too observed DLBCL to be the most common subtype comprising 29.3%, 26.4% and 26% of cases respectively. Padhi et al.[27] and Mustaq et al.[28] reported incidence to be high as 69% and 76% respectively.

Table-7 Comparision of DLBCL with various study

Study	Percentage
Sengar et al.[24](2011)	42%
Sharma et al.[23](2014)	46.80%
Present study	48.93%

Conclusion

- Lymphoma are the heterogenous group of neoplasm involving the lymphnodes.
- From our study it was concluded from histomorphology that the incidence of Non Hodgkin's lymphomas is more than the Hodgkin's lymphoma.
- Our diagnosis of hematoxyllin and eosin stained sections correlated with immunohistochemistry in all the cases.
- By immunohistochemistry it was concluded that B-cell Non Hodgkin's lymphomas were more common compare to T-cell Non Hodgkin's Lymphomas.
- It was also concluded from immunohistochemistry that diffuse large

B-cell lymphomas are the most common type of B-cell Non Hodgkin's Lymphomas.

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