Distribution of Lymphoma Subtypes in Bihar—Analysis of 518 Cases Using the WHO Classification of Lymphoid Tumors (2017)

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Abstract

Introduction The prevalent spectrum of the major subtypes of lymphoma varies across geographical regions. Through this study we aim to study the distribution of lymphoma in the state of Bihar by studying the immunophenotypic features and classifying them according to World Health Organization (WHO) 2017. To the best of our knowledge, this is the first study of this type from Bihar

Patients and Methods All the cases diagnosed as lymphoma between January 2016 and June 2019 in the Department of Pathology Mahavir Cancer Sansthan were included in the study. The cases were reviewed by two pathologists and diagnostically difficult cases were referred to higher center for opinion. A total of 518 cases were diagnosed as lymphoma.

Results B cell lymphomas formed 79.1%, whereas T cell lymphomas formed 16.2% of the total. Hodgkin lymphoma (HL) was seen in 19.6% cases. Among the non-Hodgkin lymphoma (NHL), diffuse large B cell lymphoma was the most common subtype (58% of all NHLs). Follicular center-cell lymphomas, B cell small lymphocytic lymphoma, mantle-cell lymphoma, and marginal zone B cell lymphomas (including mucosa-associated lymphoid tissue lymphomas) amounted to 2.1, 6.9, 4.5, and 0.95%, respectively. Among the T cell lymphomas, T cell lymphoblastic lymphoma, anaplastic large-cell lymphomas of T/null-cell type, and other nodal peripheral T cell lymphomas accounted for 4.7, 8.1 and 6.6% of all cases, respectively.

Conclusions The prevalence of lymphoma subtypes in India is different from the rest of the world. We have analyzed the distribution of lymphomas in Bihar and compared it with other studies from India. Follicular lymphoma and mantle-cell lymphoma are less common in India compared with the west. Peripheral T cell lymphomas and T/NK-cell lymphomas of nasal types are less prevalent than some Asian countries but are more prevalent than the west. T cell lymphoblastic lymphoma and anaplastic large T/null cell lymphoma are more common in India.

Keywords
- epidemiology
- lymphoma
- WHO 2017

Introduction

Lymphoma accounts for up to 3% of all malignancies and the estimated incidence of non-Hodgkin lymphoma (NHL) worldwide is around 5 cases per 100000.[1] It is more common in developed countries, but the incidence is increasing in India.[2] NHL is the 8th most commonly diagnosed cancer in men and the 11th in women.[3]

There are notable variations in the distribution of various types of lymphoma in different geographical regions. To the best of our knowledge, this is the first study analyzing the frequency of various subtypes of lymphoma prevalent in the state of Bihar.

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Patients and Method

Mahavir Cancer Sansthan (MCS) is a 400 bedded hospital that caters to a major chunk of cancer patients in Bihar. A total of 518 patients of lymphoma were diagnosed in the Department of Pathology, MCS, in two and a half years from January 2017 to July 2019. Clinical information regarding age, sex, and site of the biopsy was noted and hematoxylin and eosin stained sections were examined. Cases diagnosed provisionally as lymphoma on light microscopy were taken up for immunohistochemistry (IHC). All the cases were reviewed separately by two pathologists (PK, ML) and the results were recorded with consensus. Some cases, which were reported as lymphoma on light microscopy but turned out reactive after examining the excision biopsy specimen or application of IHC (like Kikuchi disease, Castleman disease, Rosai-Dorfman disease, and reactive lymphadenopathy), were excluded from this study. Plasmacytoma and multiple myeloma cases were also not included in this study.

The panel of antibodies used for IHC included CD2, CD3, CD5, CD7, tdt, CD10, CD15, CD19, CD20, CD23, CD30, CD45, CD56, CD117, CD1A, BCL2, BCL6, cyclin D1, K_i-67, and ALK-1. Panel of antibodies used in a given case was dependent on the morphological evaluation and varied from 5 to 11. IHC was performed based on horseradish peroxidase polymer chain two step indirect technique. Antigen retrieval was done using pressure cooker with Tris-EDTA buffer (pH-9.0).

Some cases that were difficult to diagnose with the above set of IHC markers or those that could not reach a common conclusion by the two pathologists were referred to a higher center for diagnosis where additional markers were used as per need (e.g., PD1, SOX11, LEF1, MUM1, TIA1, CD21, CD4, CD8, and in situ hybridization for Epstein–Barr virus encoded RNA [EBER-ISH],c-MYC)

Results

HL was reported in 102 out of 518 cases (19.6%). Age and sex distribution has been described in ►**Table 1**. All the cases were classical HL except for three cases that were nodular lymphocyte predominant type of HL. All the cases of HL presented with lymphadenopathy.

Approximately one-third of cases of NHL belonged to the 40 to 60 years age group. The disease was thrice more common in males as compared with females (►**Table 1**).

Twenty-nine percent of NHL were extranodal with the most common site being the gastrointestinal tract. Oropharynx and oral cavity were the next most common sites (►**Table 2**).

B cell lymphoma was the largest group comprising ~79% of cases. Diffuse large B cell lymphoma (DLBCL) was the most common lymphoma diagnosed (►**Table 3**). These lymphomas presented with a high proliferation index (ki67 more than 50%). CD30 was applied whenever the morphology was anaplastic and three cases were CD30 expressing DLBCL. There were four cases of T cell rich large B cell lymphoma (TCRLBCL) type of DLBCL. These cases showed diffuse effacement of architecture of the lymph node with a predominant CD3 positive T cell population and singly scattered large B cells expressing CD20, Pax5, and negative for CD30. Whenever these large B cells had a tendency to form sheets, these cases were excluded from TCRLBCL group and kept in DLBCL.

Table 2 Extranodal presentation

	Site of extranodal presentation	Frequency, *n* (%)
1	Gastrointestinal tract	58 (37.6%)
2	Oral and oropharynx	41 (26.6%)
3	Soft tissues	18 (11.6%)
4	Nose and nasopharynx	11 (7.1%)
5	Mediastinum	06 (3.8%)
6	Testis	05 (3.2%
7	Breast	03 (1.9%)
8	Bone	03 (1.9%)
9	Parotid	02 (1.29%)
10	Liver	02 (1.29%)
11	Orbit	02 (1.29%)
12	Kidney	01 (0.6%)
13	Vagina	01 (0.6%)
14	Ovary	01 (0.6%)

Table 1 Age and sex distribution

Hodgkin lymphoma—age and sex distribution (M:F = 2.9:1)					Total, *n* (%)
Age group	Male, *n* (%)		Female, *n* (%)		
0 -25	49	48	17	16.6	66(64.7)
26–50	22	21.5	07	6.8	29 (28.4)
> 50	05	4.9	02	1.9	07(6.8)

B and T cell non-Hodgkin lymphomas—age and sex distribution (M:F= 3.08:1)									
Age group	B cell lymphoma				T cell lymphoma				Total
	Male, *n* (%)		Female, *n* (%)		Male, *n* (%)		Female, *n* (%)		*n* (%)
0–20	51	12.2	06	1.4	21	5	08	1.9	86 (20.6)
21–40	57	13.7	24	5.7	16	3.8	01	0.2	98(23.5)
41–60	83	19.9	26	6.2	20	4.8	09	2.1	138(33.1)
Above 60	57	13.7	27	6.4	10	2.4	01	0.2	95(22.8)

Table 3 Frequency of lymphoma subtypes

A.	B-cell lymphoma subtype based on WHO 2017	No. of cases	% of NHL
1.	Precursor B cell lymphoblastic lymphoma	10	2.3
2.	Diffuse large B cell lymphoma	244	58.2
3.	Small lymphocytic lymphoma	29	6.9
4.	Mantle cell lymphoma	19	4.5
5.	Follicular lymphoma	09	2.1
6.	Marginal zone B cell lymphoma of MALT type	04	0.95
7.	T cell rich large B cell lymphoma	04	0.95
8.	Burkitt lymphoma	03	0.71
9.	Nodal marginal zone lymphoma	02	0.47
10.	Hairy cell leukemia/lymphoma	02	0.47
11.	Plasmablastic lymphoma	01	0.23
12.	Splenic marginal zone lymphoma	01	0.23
	Total		
B.	**T cell lymphoma subtype based on WHO 2017**	**No. of cases**	**%of NHL**
1.	Precursor T cell lymphoblastic lymphoma	20	4.7
2.	Anaplastic large cell lymphoma	34	8.1
3.	Peripheral T cell lymphoma NOS	28	6.6
4.	Angioimmunoblastic T cell lymphoma	03	0.71
5.	Follicular T cell lymphoma	01	0.23
6.	Extra nodal NK/T cell lymphoma nasal type	01	0.23
C.	**Follicular dendritic cell sarcoma**	01	0.23

Twenty-nine cases (6.9%) presented with small lymphocytic lymphoma (SLL). All the cases were above 50 years of age. The neoplastic B cells expressed CD5 and CD23 and were negative for CD10 and cyclin D1. Two cases with diagnostic difficulty showing weak to absent CD5 expression were sent to the higher center for diagnosis. These cases were LEF1 positive. Mantle cell lymphoma was reported in 4.5% of all NHL cases. They were composed of small uniform cells with cleaved or angulated nuclei without nucleoli. These cases had a low proliferation index and were expressing cyclin D1. SOX11 was used in cyclin D1 negative cases. Nine cases of follicular lymphoma (FL) were reported. These cases expressed CD10 and BCL2 and had a low proliferation index. Grading of the tumor based on the number of centroblasts was done. Five cases were grade 1 and four cases were grade 2. Marginal zone B cell lymphoma of mucosa-associated lymphoid tissue (MALT) type was seen in four cases, all of them in the stomach. These were low-grade B cell lymphomas with lack of CD5 and CD10 expression and strong immunoglobulin M (IgM) expression. Two cases of nodal marginal zone lymphoma were also reported having a similar immunophenotype. Three cases of Burkitt lymphoma, one presenting in the mandible and two in the gastrointestinal tract were seen. These cases had a very high proliferation index (95–100%), expressed BCL6, CD10, c-MYC, and were negative for BCL2. All cases with a Burkitt like morphology were sent to the higher center for IHC. We received the bone marrow biopsy of two cases of hairy cell leukemia/lymphoma (HCL). The sections revealed diffuse interstitial infiltration by tumor cells in sheets having abundant cytoplasm. Both the cases presented with typical hepatosplenomegaly, and hairy cells in the peripheral blood, diagnosed on flowcytometry as having the characteristic immunophenotype including expression of CD25 and CD103.

One case of plasmablastic lymphoma was diagnosed in a 65-year-old lady presenting with a typical presentation in the buccal mucosa, eroding the right maxillary alveolus. Biopsy showed sheets of immunoblast like cells, with high proliferation index, expressing CD38, CD138, MUM1, and immunonegative for CD30, CD3, and CK. There was a lambda light chain restriction and EBER-ISH was negative (►**Fig. 1**).

Another rare case of splenic marginal zone lymphoma (SMZL) was diagnosed in a 80-year-old male with massive splenomegaly. It was reported as prolymphocytic leukemia on peripheral smear. Bone marrow biopsy showed diffuse involvement by a low-grade lymphoproliferative disease. The tumor cells were Cd 20 positive and CD5, CD10, CD23, LEF1, cyclin D1, Sox11, and IgD negative. Ki67 was less than 5% (►**Fig. 2**).

Among the T cell NHL, the most common group comprising 8% cases was anaplastic large-cell lymphoma (ALCL). The presenting age group ranged from 20 years to 60 years. All the cases expressed CD30 and two thirds expressed Alk-1. The second most common group of NHL was peripheral T cell lymphoma not otherwise specified (PTCL NOS) accounting for 6.6% cases. This was a heterogenous group of T cell lymphoma expressing the T antigens CD3, CD5, CD7, CD2 in variable proportions and always negative for follicular helper

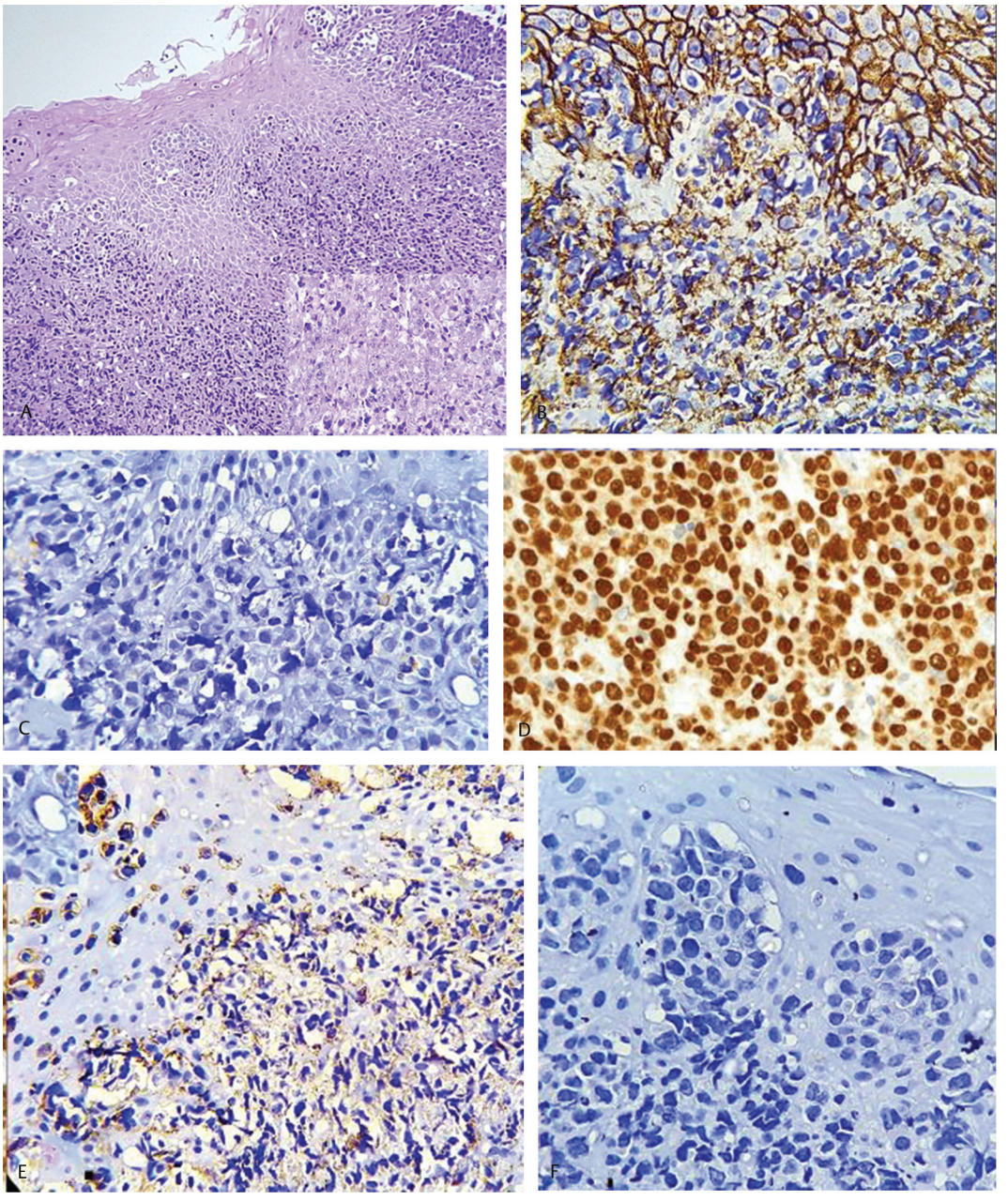

Fig. 1 Plasmablastic lymphoma: (**A**) Photomicrograph showing infiltration of the oral mucosa by large immunoblastic cells having prominent nucleoli; hematoxylin and eosin 10x (inset—40x**). (B)** CD138 positive cells, overlying squamous lining, as positive internal control. (**C**) CD20 negative tumor cells. (**D**) CD38 expressing tumor cells. (**E**) very high Ki67 proliferation index. (**F**) CD30 is not expressed by the tumor cells.

T cell markers, CD4, CD10, Bcl6, and PD1. There was one case expressing CD3, CD5, CD4, and negative for CD7, CD10, CD2 with aberrant expression of CD20 but the tumor cells were Pax5 negative. Maximum cases belonged to 50 to 70 years of age group.

Of the 20 cases of T cell lymphoblastic lymphoma, three involved the mediastinum, three in the bone, and the remaining in lymph node and soft tissues. All the cases were seen in patients less than 30 years of age. The tumor cells had a high proliferation index (> 70%) and expressed nuclear antigen tdt along with T specific antigens. Among the follicular helper T cell lymphomas, three cases of angioimmunoblastic T cell lymphoma (AITL) and one case of follicular T cell lymphoma were diagnosed. Lymph nodes of AITL showed a polymorphous population of cells with numerous high endothelial venules. Paracortical aggregates of medium sized T cells having pale cytoplasm and prominent follicular dendritic meshwork highlighted by CD23 and CD21 were noted. The tumor cells had the phenotype of a follicular helper T cell, expressing CD10, bcl6, and PD1. We had one case of follicular T cell lymphoma (FTCL) in a 60-year-old male presenting with lymphadenopathy. This lymphoma showed predominantly a follicular pattern of involvement of lymph node by neoplastic T cells expressing CD4 and PD1. These cells were negative for CD20. Follicular dendritic cell meshwork was not expanded, as observed by CD23 stain (►**Fig. 3**).

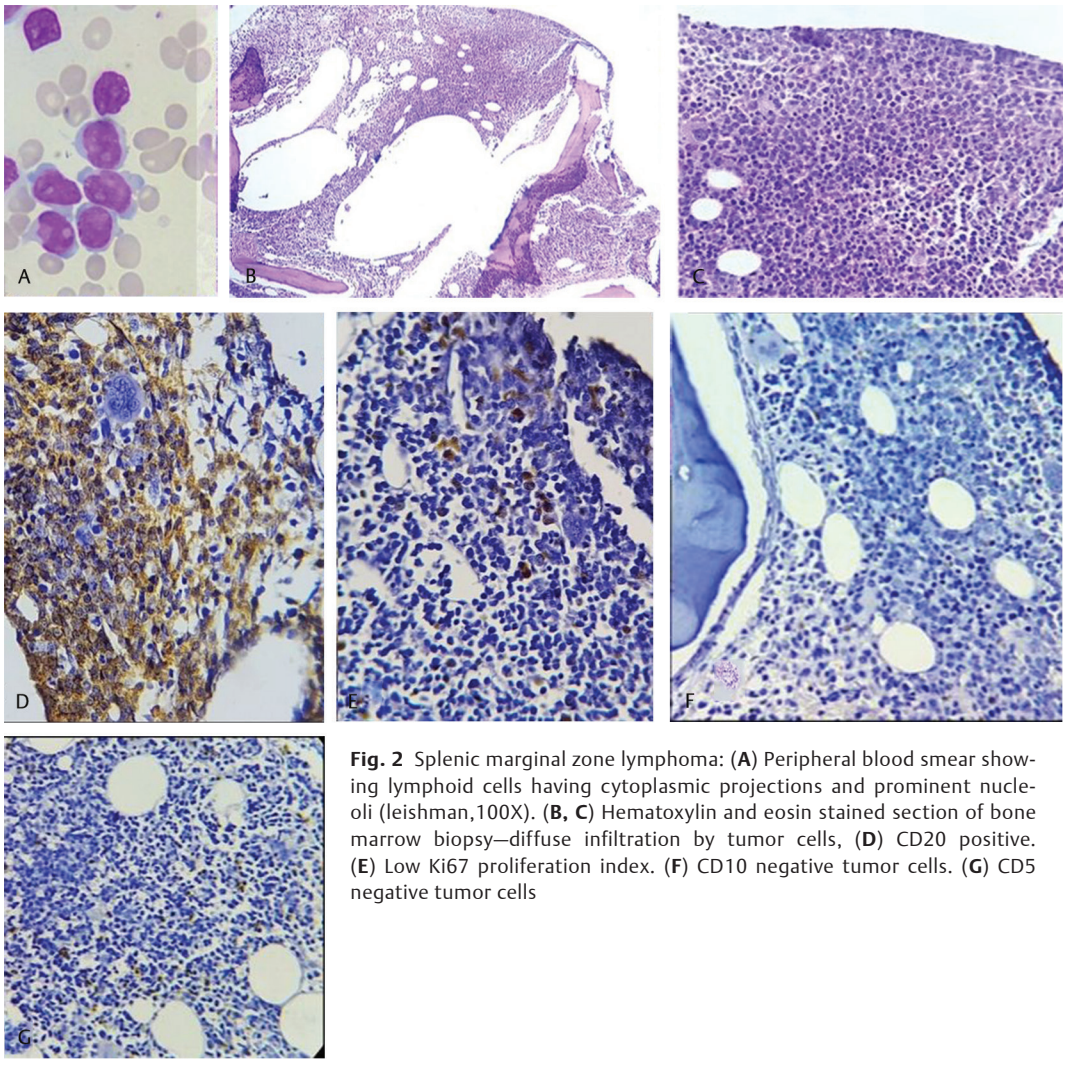

Fig. 2 Splenic marginal zone lymphoma: (**A**) Peripheral blood smear showing lymphoid cells having cytoplasmic projections and prominent nucleoli (leishman,100X). (**B, C**) Hematoxylin and eosin stained section of bone marrow biopsy—diffuse infiltration by tumor cells, (**D**) CD20 positive. (**E**) Low Ki67 proliferation index. (**F**) CD10 negative tumor cells. (**G**) CD5 negative tumor cells

One case of extranodal NK/T cell lymphoma nasal type (ENKTCL) was also reported. It was seen in a 62-year-old female presenting with ulcerative soft tissue mass involving the nostrils, extending into anterior nasal concha, without bony erosion. This was misdiagnosed as squamous cell carcinoma on biopsy at a private laboratory. Wide local excision of the distal nasal tumor with the nose, upper lip, nasal bone, and premaxilla was done. Sections from the tumor revealed infiltration by a population of small lymphoid cells having irregular nuclear foldings, with prominent angiocentric growth, ulceration, and necrosis. These tumor cells expressed CD56, CD7, and CD2, patchy CD3, CD4, TIA1, and EBER (by in situ hybridization), and were immunonegative for CD20, CD138, CD38, CD5, and CD8 (►**Fig. 4**).

We had one case of follicular dendritic cell sarcoma (FDCS). It was seen in retroperitoneal mass of a 40-year-old male. The biopsy sections revealed sheets of epithelioid cells with sprinkling of lymphocytes. These cells expressed CD21, CD23, and CD35 and were negative for Cd45, Cd30, Cd20, S100 protein, CK, EMA, desmin, chromogranin, CD34, Ckit, DOG1, and ER.

Discussion

The classification of lymphoma is evolving over the years with the expansion of our knowledge and the development of better techniques for its detection and characterization. The World Health Organization (WHO) classification of tumors has now become a household name among pathologists, continuously updating us about the newer entities. In the present study, we have classified lymphoma based on the recent 2017 edition of WHO.[4] The incidence of lymphoma is higher in the developed nations. In India also, lymphoma is more common in the urban areas as compared the rural.[2,5] Urban population has a higher socioeconomic status and their lifestyles also lean toward the west whereas the rural population has a more traditional lifestyle. This variation in socioeconomic and environmental factors in the rural and urban areas may be responsible for the difference in the occurrence of lymphoma.[6] The global burden of lymphoma is increasing and the disease is more common in males. Male to female ratio of incidence of HL and NHL, worldwide, is 1.6:1 and 1.3:1, respectively.[7] Two studies from south India

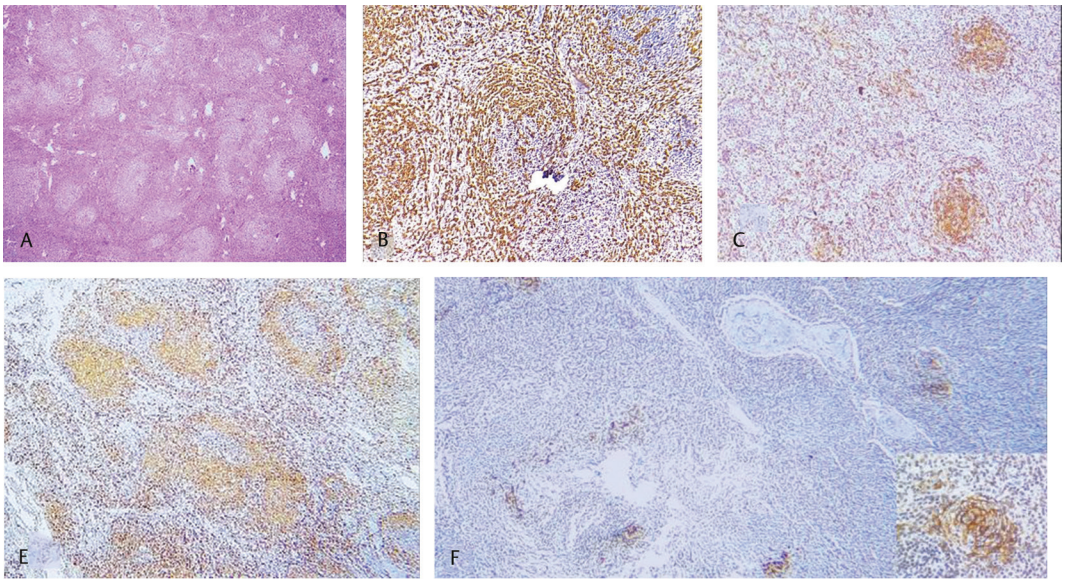

Fig. 3 Follicular T cell lymphoma: (**A**) Follicular pattern of involvement of the lymph node **(B).** CD3 positive follicular T cells (**C**). CD20 positive remnant germinal centers (40x**). (D)** PD1 positive in the neoplastic follicular T cells (10x). (**E**) Follicular dendritic meshwork not expanded as evidenced by CD23 stain (10x, inset 40x).

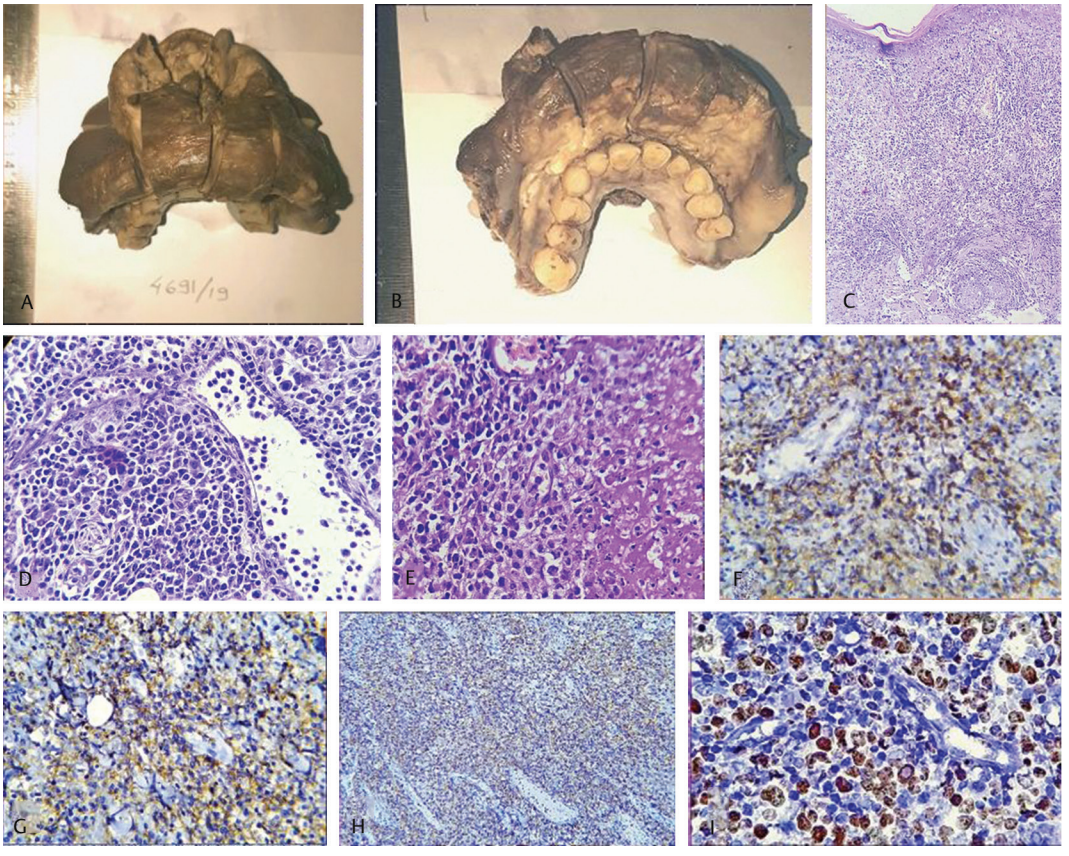

Fig. 4 NK/T cell lymphoma, nasal type: (**A, B**) Gross images of partial maxillectomy specimen. (**C**) Section showing infiltration by tumor cells and the overlying mucosa (hematoxylin and eosin [H&E],10X). (**D**) Angiocentric growth of tumor cells infiltrating the blood vessel wall (H&E, 40X). (**E**) Necrosis, apoptotic bodies (H&E, 40X). (**F**) CD7 positive neoplastic natural killer/T cells. (**G**) CD56 positive tumor cells. (**H**) TIA1 positive tumor cells. (**I**) In situ hybridization for Epstein–Barr virus encoded RNA was positive in the tumor cells.

have reported a slightly higher incidence in males compared with the global data. According to these studies, male:female ratio in HL is 2.5:1, while that in NHL is 2.2 to 2.8:1.[8,9] In our study, both HL and NHL are thrice as common in males as in females. One study from eastern India reports results similar to our results.[10] Maximum cases of HL in our study were less than 25 years of age (64.7%) and those of NHL belonged to the age group of 41 to 60 years. This is in concordance

with the other Indian studies.[8-10] Extranodal presentation is common in some lymphomas like DLBCL, Burkitt lymphoma, MALT lymphoma, and PTCL. Studies from Western countries have shown that extranodal NHL are seen in 24 to 48% of all NHL.[11] Our neighboring countries like China and Japan have even higher incidence of extranodal lymphoma ranging from 44 to 60% as per literature.[12,13] This may be because of the higher prevalence of extranodal NK/T cell lymphoma in these countries compared with India. Two studies from south India, Padhi et al and Mishra et al have reported the incidence of extranodal lymphoma to be 22 and 22.6% of all NHL, respectively.[14,15] Padhi et al reported the central nervous system as the most common site, followed by the gastrointestinal tract, whereas Mishra et al reported head and neck and gastrointestinal tract as the most common site. In our study, 29% patients presented with extranodal disease and the most common sites were the gastrointestinal tract and head and neck. We do not have a neurosurgery unit at our center and all such cases are outsourced and central nervous system lymphoma has not been included in this study. The results of the study from eastern India by Mondal et al are similar to our study with 27.7% extranodal presentation.[10]

►**Table 4** lists the frequency of various types of NHL in India and its comparison with USA and China. DLBCL is the most common lymphoma in India as well as other countries with 40 to 50% of cases. We reported 59% DLBCL in our study. One of the largest studies from India by Arora et al reported FL as the second largest group among the NHL with 10.9% cases, followed by SLL at 4.1% (►**Table 4**). Our study differs slightly, with the second most common NHL being SLL (6.9%), followed by mantle cell lymphoma (4.5%). FL forms only 2.1% of all NHL in our study. This maybe because the two studies from India are done in south India that is socioeconomically more developed compared with Bihar. FL is known to be more common in countries with higher socioeconomic status. In the West, SLL is the second most common lymphoma (21.9%) after DLBCL, whereas in China only 4.6% cases are SLL. The frequency of other indolent B cell NHL in our study was in concordance with other studies from India (►**Table 4**). Seven percent cases were lymphoblastic lymphoma in the present study. A similar frequency has been found in other studies from India as well as other parts of world.[8,11,12,16] An average of 2 to 3% cases of Burkitt lymphoma has been reported in Indian studies; in our hospital, however, only 0.7% cases were reported. This is because of the fact that c-MYC translocation study could not be done in some cases suspicious of BL and such cases have been excluded from this study.

The incidence of T cell NHL appears to increase worldwide as we move from West to East. About 26.1% T cell NHL was reported in China, while in the USA it was found in only 7% cases. The frequency of T cell NHL reported in India is ~20%[8,16]. In the present study, also 21% NHL were of T cell type. Naresh et al have reported T lymphoblastic lymphoma as the commonest T cell NHL. They might have included the cases presenting with leukemia, without lymphadenopathy in their study giving a higher incidence. In our study, we have only included the cases with lymphoma, similar to Arora et al. But we got slightly higher incidence of TLBL (4.7%) compared with them (2.2%). Among other T cell NHL,

Table 4 Distribution of lymphoma across the world (in percentage)

Type of lymphoma	India			USA	China
	Arora et al,[8] 2013 (n = 5,115)	Naresh et al,[16] 2000 (*n* = 2,773)	Present study (n = 518)	Morton et al,[11] 2006 (*n* = 1,14,548)	Yang et al,[12] 2011 (*n* = 6,382)
Precursor B and T cell lymphoblastic lymphoma (B+T)	0.4 +2.21	0.6 + 6.06	2.3 + 4.7	3.75+ 1.09	5.2
Diffuse large B cell lymphoma + TCRLBL	46.9	33.8	59	31.2	41.2
Small lymphocytic lymphoma	4.1	5.6	6.9	21.9	4.6
Mantle cell lymphoma	1.6	3.4	4.5	2.18	3.15
Follicular lymphoma	10.9	12.6	2.1	13.8	5.8
MALT lymphoma	2.4	6.1	0.95	4.19	6.3
Burkitt lymphoma	3.4	1.8	0.71	1.4	1.9
Nodal marginal zone lymphoma	0.8	1.9	0.47	–	0.09
Hairy cell leukemia/lymphoma	0.8	0.01	0.47	1.44	–
Plasmablastic lymphoma	–	–	0.23	–	–
Splenic marginal zone lymphoma	0.4	0.2	0.23		0.35
Anaplastic large cell lymphoma	5.1	4.1	8.1	1.11	3.53
Peripheral T cell lymphoma NOS	5.9	1.9	6.6	3.27	3.99
Angioimmunoblastic T cell lymphoma	2.2	1	0.71	0.23	3.33
Follicular T cell lymphoma	–	–	0.23	–	–
Extra nodal NK/T cell lymphoma nasal type	0.9	0.7	0.23	0	17

PTCL NOS and ALCL were the most common in India.[8,16] A similar trend was seen in our study as well. AITL is the next common T cell NHL in India with the reported incidence of 1 to 2%. In the present study, three cases (0.71%) were AITL. While ENKTCL is rare in the west, it is very common in the eastern part of the world.[12,17] In China, it is the commonest T cell NHL (►**Table 4**). In India, the incidence of ENKTCL has been reported to be 0.7 to 0.9%. However, in our study there was only one case (0.23%). We also reported one rare case of FTCL. FTCL was kept in the PTCL NOS group in WHO 2008,[18] but is a separate entity in the recent 2017 edition[4] along with other tumors arising from follicular helper T cells. One case of FDCS was also included in this study as it also arises from the dendritic cells of the lymph node and presents like a lymphoma. Other studies from India have not included FDCS and thus the incidence has also not been mentioned. In one study from south India, only 10 cases were reported over a period of 5 years.[19]

Conclusion

This study documents the frequency of different types of lymphoma in Bihar. Most common lymphoma is DLBCL. Incidence of FL is much less common compared with the west. T cell NHL is more common than the West, but is less prevalent than some Asian countries. In this study, we have also reported the frequency of some rare cases like HCL, SMZL, ENKTCL, FTCL, and FDCS in Bihar.

Abbreviations: MALT, mucosa-associated lymphoid tissue; NHL, non-Hodgkin lymphoma; NK, natural killer; NOS, not otherwise specified; WHO, World Health Organization.

Abbreviations: MALT, mucosa-associated lymphoid tissue; NK, natural killer; NOS, not otherwise specified; TCRLBCL, T cell rich large B cell lymphoma.

Conflict of Interest
None declared.

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