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RESEARCH ARTICLE

NON-HODGKIN'S LYMPHOMA: IMMUNOHISTOCHEMICAL ANALYSIS WITH CLINICOPATHOLOGICAL CORRELATION

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ABSTRACT

Objective: Histomorphology and immunohistochemistry are essential tools for evaluation and classification of lymphoid malignancies. The present prospective study was conducted with the aim to categorize various Non-Hodgkin lymphoid malignancies in lymph node and extranodal biopsies using routine H and E staining and IHC markers based on WHO Classification system 2016 and to study their clinicopathological correlation.

Material and methods: The present study was conducted in Department of Pathology at Pt. B D Sharma PGIMS, Rohtak. Hundred cases of Non Hodgkin's lymphoid malignancy (lymph node and extranodal biopsies) were included in the study. Various histomorphological changes were examined on routine H&E. Cases with provisional diagnosis of lymphoma were further submitted to immunohistochemical staining for a panel of lymphoma markers and classified accordingly.

Results: Maximum number of patients were in the age group of 61-70 years (26%). Males were more commonly affected (55%) with a male: female ratio of 1.2:1. Study included 57 cases with nodal and 43 cases with extranodal involvement. Among nodal, cervical (40.4%) was the most common site and among the extranodal, GIT was (27.9%) the most affected site. A significant association was found between presence of B symptoms with male gender and extranodal sites ($p<0.05$). B Cell type was the commonest type of Non Hodgkin's lymphoid malignancy observed in the study (80%). The most consistent IHC marker for B cell lymphoma was PAX5 followed by CD20 and CD19 whereas CD5 was the most consistent IHC marker for T cell lymphoma followed by CD3.

Conclusion: The distribution of NHL subtypes in India shows important differences with those from the rest of the world. PAX 5 can be used as a universal single pan B cell marker and CD5 as a pan T cell marker in resource poor setting.

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INTRODUCTION

Lymphoid malignancies comprise a diverse group of different morphological and clinical syndromes. Their incidence is increasing rapidly with about 1,00,000 new cases being diagnosed each year in the United States. The reasons for increasing incidence are multiple, ranging from the increasing elderly population, greater accuracy of diagnosis and unknown environmental factors. Lymphoid neoplasms comprise Non Hodgkin′s Lymphoma (NHL) and Hodgkins Lymphoma (HL) (Cerny and Gillessen, 2002). Non-Hodgkin's lymphoma (NHL) is characterized by neoplastic proliferation of cells of lymphoid tissue i.e. lymphocytes, histiocytes and their precursors (Muzaffar *et al.,* 1997).

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It usually presents as painless, localized or generalized enlargement of lymph nodes with or without hepatosplenomegaly. However, it may present as a tonsillar, mediastinal or abdominal mass, or as localized or generalized lesions involving every organ or organ system (Chang *et al.,* 2015). The gastrointestinal tract is the most common extranodal site for NHL and the other sites include testis, bones, eyes, brain, heart, white blood cells, skin and kidneys (Akhter *et al.,* 2012). Our ability to classify patients with NHL into clinically useful groupings has steadily improved as our understanding of the biology of the immune system has advanced. The use of histopathology to diagnose malignancies and the discovery of the Reed Sternberg cell early in the 20th century allowed a distinction between Hodgkin disease and the other lymphomas grouped together as Non-Hodgkin's Lymphoma (Armitage, 2005). NHL is increasing in incidence worldwide and is the 11th most common cause of cancer. It is usually fatal, with a 5 year survival rate of less than 35 percent (Yeole, 2008).

Lymphomas represents one of major health problems all over the world. NHLs are slightly more common in developed countries (50.5 % of cases worldwide) with rates highest in Australia and North America, intermediate in Europe (except Eastern Europe) and Pacific islands and relatively low throughout Asia and Eastern Europe (Parkin *et al.,* 2005). In Asia, the incidence of NHL has increased in recent decades. The age-adjusted incidence rates for NHL in men and women in India are 2.9/100,000 and 1.5/100,000, respectively. These are about one fourth of the incidence rates reported from Western Europe or North America. Within India, the incidence is several-fold higher in urban cancer registries compared to rural areas; the incidence being higher in metropolitan cities and Indian immigrants suggesting that urban lifestyles and economic progress may increase the cancer incidence. Compared to developed nations, the key differences in the presentation in India include: median age of 54 years (almost a decade less), higher male to female ratio, higher proportion of patients with B-symptoms (40–60 vs. 20–30%), poor ECOG performance status (≥ 2) at diagnosis (50 vs. 20–30%), higher frequency of diffuse large B-cell lymphomas (60–70 vs. <40%), lower frequency of follicular NHL (<20 vs. 30–40%) and T-cell type in 10–20 vs. <10%.

The estimated mortality rate due to NHL is higher in India than in North America and Western Europe. Although the incidence rate of NHL in India is lower compared to that in developed countries, the mortality rate is nearly same. According to the Globocan (2012) data, the ratio of mortality to incidence in India is 69.7%. This reflects the poor overall 5-year survival below the global average.[8] Patients with primary or secondary immunodeficiencies are at greater risk for lymphoma. These patients often show diffuse NHL morphologies, extranodal disease, and association with Epstein Barr virus (EBV) infection. Various occupational, environmental, and chemical agents have also been analyzed as risk factors for NHL (Bukhari *et al.,* 2015). Lymphomas cause B symptoms that include drenching night sweats, unexplained weight loss, fever and severe itching. Patients having B symptoms show a more severe condition than asymptomatic patients with the same cancer stage, tumor location or size. Onset of B symptoms at the time of diagnosis suggests that lymphoma is progressing (El-Esawy *et al.,* 2015).

The foremost means of diagnosis of NHL is Hematoxylin and Eosin based morphological examination though immunohistochemistry is capable to differentiate the lymphocytes into T and B cell (Krenacs *et al.,* 2002). Lymphomas can be diagnosed from a number of different specimens like lymph nodes, bone marrow aspirates, trephine biopsy cores and peripheral blood as well as other fluids such as cerebrospinal fluid (CSF), ascitic fluid and pleural aspirates, depending on the presenting clinical features. Due to overlap of morphologic features and antigen expression, undifferentiated, anaplastic, and certain hematolymphoid malignancies can constitute a diagnostic challenge. In most cases, immunohistochemical characterization using a panel of antibodies can resolve cell lineage like antibodies to CD45 (hematolymphoid), cytokeratin (carcinoma), vimentin (sarcoma), and synaptophysin (neuroendocrine). The initial immunohistochemical panel for lymphomas include CD20 (B-cells), CD3 (T-cells), CD138 (plasma cells) and CD30 (Anaplastic B- and T-cell lymphomas). Multiple antibodies are then utilized for subtyping the B- and T-cell lymphomas to make a specific diagnosis (Desouki *et al.,* 2010).

MATERIALS AND METHODS

Hundred cases of NHL (nodal and extranodal) formed the study group. A complete clinical and haematological profile of the patient including age, gender, anatomic location, number of lymph nodes involved, extranodal sites and presence or absence of B Symptoms were recorded. Only confirmed cases of NHL on biopsy from nodal and extranodal sites were included in this study. Cases in which adequate tissue sample or complete clinical profile of patient is not available, were excluded from the study. All the biopsy specimen submitted with clinical diagnosis of lymphoma were processed by routine histological technique for paraffin embedding and stained with hematoxylin and eosin (Bancroft and Layton, 2012). Cases with provisional diagnosis of lymphoma were further submitted to immunohistochemical staining for a panel of lymphoma markers and classified accordingly. IHC was performed as follows: Paraffin sections (3-5 µm in thickness) mounted on slides with suiTABLE tissue adhesive were processed for deparaffinization in xylene and rehydration through graded alcohols. Endogenous peroxidase enzyme was blocked by using 3% hydrogen peroxidase for 15-20 minutes. Antigen retrieval was done with fully automated system-DAKO PT Link. This system requires pre-treatment with heat induced epitope retrieval. Sections then incubated with the monoclonal antibody (pre-diluted) overnight at 4°C. Then sections were rinsed with TBS solution followed by incubation with the secondary antibodies. The reaction was visualized using DAB (3,3′-Diaminobenzidine), and nuclei were counterstained with hematoxylin. Positive and negative controls were run with each batch of IHC stain. Positive control were sections from tonsil and splenic tissue. Negative control was obtained by substituting the primary antibody with an antibody of nonspecific positivity.

Interpretation of result

Nuclear and cytoplasmic staining of various lineage specific IHC markers was taken as positive. The results were interpreted on the basis of number of cells stained, area and intensity of staining for various markers.

Statistical Analysis

A descriptive study was carried out for all the variables included in the study. Chi-square test was used to compare the categorical values. P-value less than 0.05 was accepted as statistically significant.

RESULTS

In our study of 100 cases of Non Hodgkin lymphoid malignancies, the minimum and maximum ages recorded were 9 and 85 years respectively. Majority (n=26, 26%) of patients were in the age group of 61-70 years followed by 23 cases (23%) in age group of 41-50 years and 15 cases (15%) being in the 51-60 years age group. The average age at diagnosis was 52.31 years. The median age of cases was 55 years. Males (n=55) were predominantly affected compared to females (n=45) with M:F ratio of 1.2:1, Out of 55 males, 37 (67.3%) presented with B- symptoms, while out of 45 females, B symptoms were present in 21 (46.7%) cases. Association between male gender and B symptoms was found to be significant. (p=0.038) (Table 1). There were 57 nodal and 43 extranodal cases (Figure 1 and 2).

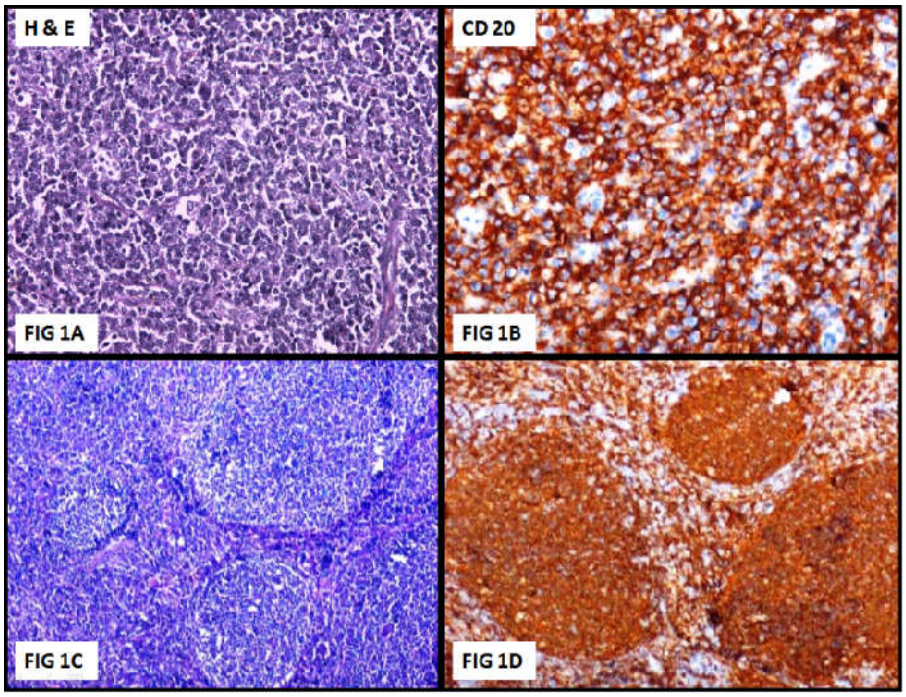

Figure 1. Photomicrograph showing Diffuse Large B Cell Lymphoma (1a&1b) and Follicular Lymphoma (1C&1D)

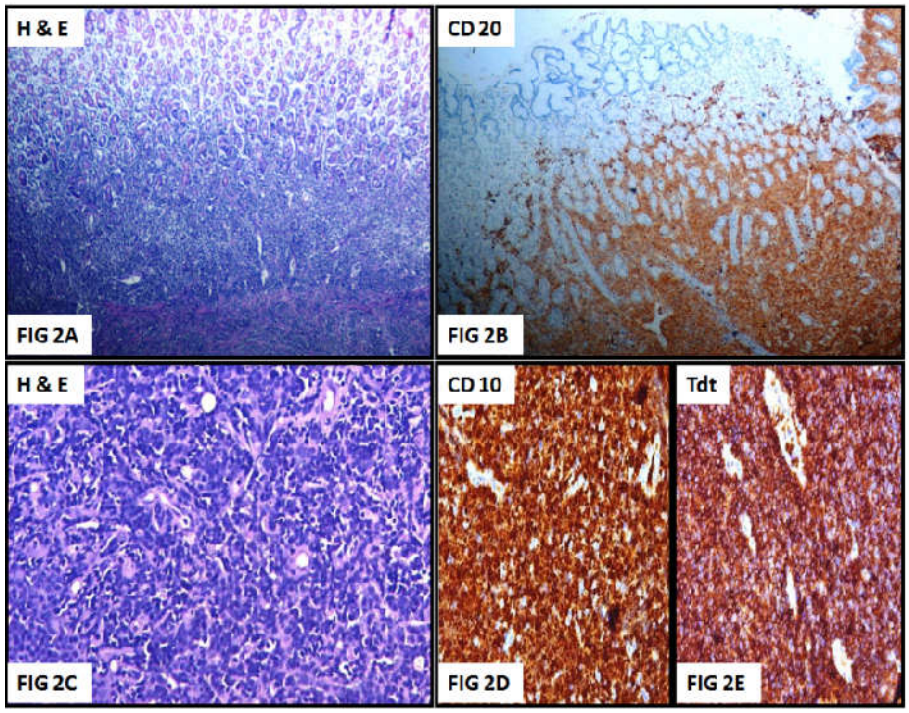

Figure 2A & 2B. Photomicrograph showing Extranodal Marginal Zone lymphoma (GIT); Figure 2C & 2D. Photomicrograph showing Primary Bone Lymphoma -Precursor B Cell Lymphoblastic Type

Out of 57 nodal cases, 23 cases were from cervical region accounting for 40.4% of cases, 17 were from inguinal region accounting for 29.8% of cases and 11 cases involved axillary area accounting for 19.3% of cases. Supraclavicular and mediastinal regions accounted for 7% and 3.5% of cases respectively. Out of 43 extranodal cases, 12 cases were from gastrointestinal tract (Figure 2A and 2B) accounting for 27.9% of cases, 6 were from waldeyer's ring accounting for 14% of cases. Bone marrow and head and neck each accounted for 11.6% of cases. Mediastinal and lower limb involvement was seen in 9.3% of cases. Skin and testes each accounted for 4.7% of cases. Vulva, pelvis and paravertebral mass each accounted for 2.3% of cases.

Among 57 nodal cases, 27 (47.4%) showed presence of B-symptoms, whereas out of 43 cases of extranodal origin, the presence of B-symptoms was seen in 31 (72.1%) patients. A significant association was seen between B symptoms and extranodal involvement. (p=0.013). (Table 2) Lymphadenopathy was observed in 68% of cases, Hepatomegaly in 60%, splenomegaly in 42% of cases and B Symptoms in 58% of cases. Non Hodgkin Lymphoma B-Cell type was the more common type constituting 80% of cases followed by T-Cell type constituting 20% of cases. (Table 3) Among the B –cell type, DLBCL (Figure 1A and 1B) was the most common subtype constituting 40% of the total B cell cases and 32% of all the 100 cases.

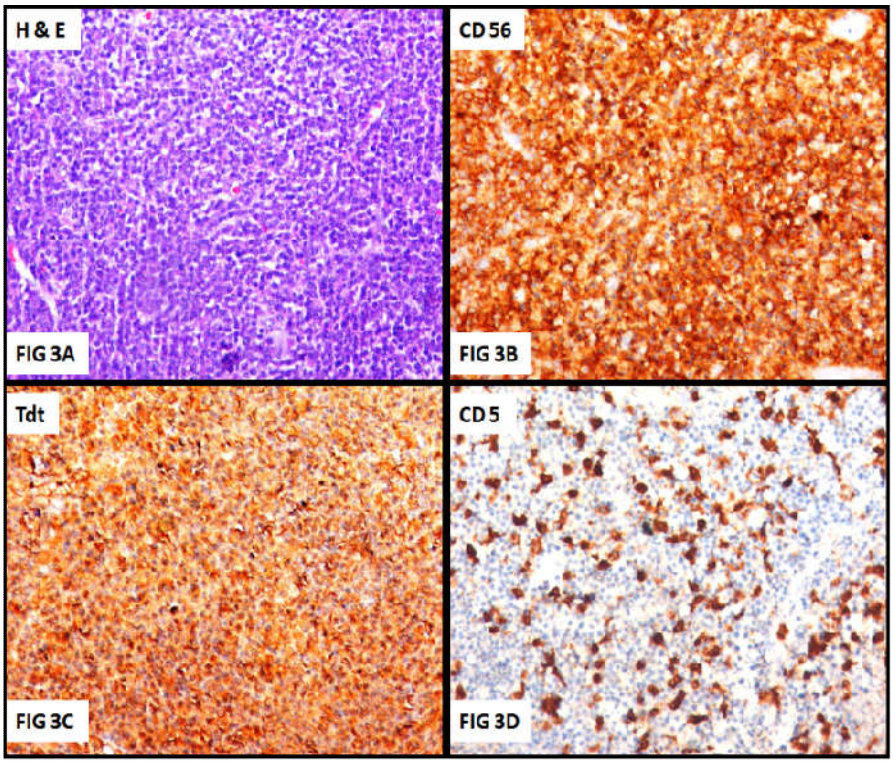

Figure 3. Photomicrograph showing Primary Intestinal NK/T Cell Lymphoma A. H&E; B. IHC CD56 (Positive); C. IHC Tdt (Positive);D. IHC CD5 (Negative)

Table 1. Frequency of B symptoms according to sex distribution (n=100)

Gender * B symptoms cross tabulation					
			B Symptoms		Total
			Negative	Positive	
Gender	F	Count	24	21	45
		% within B Symptoms	57.1%	36.2%	45.0%
	M	Count	18	37	55
		% within B Symptoms	42.9%	63.8%	55.0%
Total		Count	42	58	100
		% within B Symptoms	100.0%	100.0%	100.0%
		Chi-Square Tests			
		Value	Df	P value (significant if <0.05)	
Pearson Chi-Square		4.314	1	0.038	
N of Valid Cases		100			
b. Computed only for a 2x2 table					

Table 2. Frequency of B symptoms according to site (n=100)

Site * B Symptoms cross tabulation					
			B Symptoms		Total
			Negative	Positive	
Nodal / Extranodal	Nodal	Count	30	27	57
		% within B Symptoms	71.4%	46.6%	57.0%
	Extranodal	Count	12	31	43
		% within B Symptoms	28.6%	53.4%	43.0%
Total		Count	42	58	100
		% within B Symptoms	100.0%	100.0%	100.0%
		Chi-Square Tests			
		Value	Df	P value (significant if <0.05)	
Pearson Chi-Square		6.151	1	0.013	
N of Valid Cases		100			
b. Computed only for a 2x2 table					

Table 3. Frequency distribution of NHL cases according to cell lineage (n=100)

Type of NHL	No. of cases	Percentage
B – Cell type	80	80%
T – Cell type	20	20%
Total	100	100%

Table 4. Frequency distribution of various subtypes of NHL (n=100)

Diagnosis	No. of cases	Valid percent
Dlbcl	32	32
NHL- b cell type (nos)	26	26
NHL- t cell type	13	13
Follicular lymphoma	12	12
Small lymphocytic lymphoma	4	4
Anaplastic large cell lymphoma	3	3
Mantle cell lymphoma	2	2
Primary intestinal nk/t cell lymphoma	1	1
Precursor b cell lymphoblastic lymphoma	1	1
Marginal zone lymphoma (malt)	1	1
Lymphoblastic lymphoma - t cell type	1	1
Lennert's lymphoma	1	1
Burkitt's lymhoma	1	1
Angioimmunoblastic - t cell lymphoma	1	1
Acute lymphoblastic lymphoma	1	1
Total	100	100

Table 5. Comparison of frequency distribution of various subtypes of NHL with other studies

NHL Subtypes Frequency (%)	Present Study	Akther et al	Naz et al	Sharma et al	Naresh et al	El-Esawy et al	Bukhari et al	Devesa et al
B CELL TYPE	80	88	85.5	89.3	79.1	85	87	83.8
DLBCL	32	34	58.1	46.8	34	63.4	59	31.3
Peripheral B cell NHL – NOS	26	-	-	-	10.5		-	-
Follicular Lymphoma	12	4	9.7	6.4	12.6	10.9	5.2	13.8
SLL/CLL	4	24	8.1	17	5.6	4.4	9	21.9
Mantle cell lymphoma	2	-	1.6	12.8	3.4	1.7	2.6	2.2
Marginal Zone Lymphoma (MALT)	1	-	-	2.1	6.1	-	3.1	4.2
Acute Lymphoblastic Lymphoma	1	4	1.6	-	0.6	0.9	-	3.8
Precursor B cell LBL	1	-	-	2.1	0.6	-	1.6	-
Burkitt's Lymphoma	1	8	3.2	-	1.8	3.1	-	1.4
T CELL TYPE	20	12	14.5	10.7	15.2	15	13	16.2
Peripheral T Cell Lymphoma	13	8	1.6	-	1.9	1.5	6.8	3.3
Anaplastic Large Cell Lymphoma	3	-	11.3	2.1	4.1	11.3	3.6	1.1
Lennerts Lymphoma	1	-	-	-	-	-	-	-
Angioimmunoblastic T Cell Lymphoma	1	4	-	2.1	-	-	-	0.2
Lymphoblastic Lymphoma – T cell type	1	-	1.6	6.4	6	2.2	1.6	1.1
Primary Intestinal NK/T cell lymphoma	1	-	-	-	0.7	-	1.1	2.3

Peripheral B cell- NOS constituted 26%, Follicular lymphoma (Figure 1C and 1D) 12%, SLL 4%, and Mantle cell lymphoma constituted 2%. Marginal zone lymphoma, Acute lymphoblastic lymphoma, Burkitt's Lymphoma and Precursor B cell lymphoblastic lymphoma (Figure 2B and 2C) each constituted 1% of the total cases. Among T-cell neoplasms (Figure 3), 13% of cases were of peripheral T cell lymphomas, 3% of anaplastic large cell lymphomas and 1% each of lennerts, angioimmunoblastic, lymphoblastic and primary intestinal NK/T cell lymphoma (Table 4). Overall B symptoms were found in 58 (58%) cases, out of which 49 (84.48%) cases were of B cell and 9 (15.52%) cases of T cell type. No significant association was found between B symptoms and cell lineage (p=0.188). Almost all cases of B Cell type lymphoma, i.e. DLBCL, SLL, Peripheral B Cell Lymphoma-NOS, follicular lymphoma, mantle cell lymphoma and marginal zone lymphoma showed positive immunoexpression for CD19, CD20 and Pax-5 whereas most of the Non Hodgkin Lymphoma-T cell type were positive for CD3 and CD5 (Figure 3).

DISCUSSION

Lymphoid malignancies are a major burden to afflicted patients, medically and financially. The diagnosis of Non Hodgkin's Lymphoma is based on a constellation of clinical, laboratory features, histomorphology, immunophenotyping and, where appropriate, molecular genetic analysis. A highly selective panel of immunostains, based on the histopathological impression of the tumor, can be extremely useful to narrow the diagnostic considerations as most lymphomas are substantially defined by their immunoprofile.[12] In our present study, we attempted to categorize various Non Hodgkin lymphoid malignancies according to WHO Classification system 2016 and studied their clinicopathological correlation and observed vast number of findings compatible with various studies and few findings contrary to previous studies. We found a significant association between male gender and B symptoms (p=0.038). These observations are in concordance with the various population based studies like El-Esawy *et al.* and Naz *et al.* The higher frequency of B symptoms in male patients can be attributed to higher frequency of bone marrow involvement in males and increased incidence of predominantly higher grade NHL in males. A significant association was seen between B symptoms and extranodal involvement. (p=0.013) which can be attributed to a more aggressive tumor, tumor location, size and presentation at an advanced stage in extranodal lymphomas. In concordance to our study, El-Esawy *et al.* observed that out of total 320 cases, 190 (59.4 %) presented with nodal involvement and 130(40.6 %) had extra nodal site. However, out of 190 patients with nodal involvement, 56 (29.5%) showed the presence of B symptoms, whereas of 130 cases of extra nodal origin, the presence of B symptoms was seen in 92 (70.7%) patients. They also found significant association of B symptoms with extranodal involvement. (p=0.001) (El-Esawy and Elaskary, 2015). Similarly, Naz *et al.* observed that out of 37 patients with nodal involvement, 11 (29.7%) showed the presence of B symptoms, whereas 25 cases of extra nodal origin, the presence of B symptoms was

seen in 18 (72%) patients. Significant association (p=0.001) was seen in the presence of B symptoms with extranodal involvement (Naz *et al.,* 2011). Overall B symptoms were found in 58 (58%) cases, out of which 49 (84.48%) cases were of B cell and 9 (15.52%) cases of T cell type. No significant association was found between B symptoms and cell lineage (p=0.188). Similar to our findings, study of El-Esawy *et al.* and Naz *et al.* found no significant association between cell lineage and B symptoms. An insignificant association of B symptoms and T cell lymphomas in spite of its aggressive clinical behaviour and high grade pattern can be attributed to comparatively small number of cases included in these studies. In our study, we observed that B-Cell type NHL was more common (80%) than T-Cell type. Among the B –cell type, DLBCL was the most common subtype (40%) whereas Peripheral T cell lymphoma (13%) was the most common T-cell neoplasm. Our study was in concordance with most of the studies i.e. Akhter *et al,* Sharma et al, Naresh *et al,* El-Esawy *et al.* and Nair *et al,* where B cell lymphomas constituted the majority of lymphomas with DLBCL being the most common subtype. However, study done by Devesa *et al.* in USA shows dissimilarity where DLBCL accounted for 31 % of cases only. A higher frequency of DLBCL in our study and lower frequency of follicular lymphoma, mantle cell lymphoma and other indolent lymphomas can be attributed to younger age of patients at presentation and shorter life span as compared to western population (Table 5).

Conclusion

Lymphoma presents commonly as lymphadenopathy, fever, weight loss and hepatomegaly with a close resemblance to tuberculosis and other disorders. This may cause difficulty to practitioners for early diagnosis. A general awareness regarding the clinical manifestations of lymphoma, along with usage of advanced investigative techniques, may lead to early diagnosis of this relatively curable disease. The most consistent IHC marker for B cell lymphoma was PAX5 followed by CD20 and CD19 whereas CD5 was the most consistent IHC marker for T cell lymphoma followed by CD3. PAX 5 can be used as a universal single pan B cell marker and CD5 as a pan T cell marker in resource poor setting.

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