VOLUME - 9, ISSUE - 11, November - 2020 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Pathology

HISTOPATHOLOGICAL DIAGNOSIS OF LYMPHOMAS IN A TERTIARY CARE CENTRE IN EASTERN INDIA: THE SPECTRUM AND PITFALLS.

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ABSTRACT **Background:** There are various studies subtyping lymphomas on the basis of histopathology and immunohistochemistry (IHC) but there is sparse literature regarding the same from eastern India. **Aims and Objectives:** To analyse the subtype distribution of lymphomas on the basis of histopathology and IHC in a single tertiary care centre from eastern India. **Materials and Methods:** This was a retrospective, observational study comprising 134 cases of lymphoma over the last three years from August 2016 to July 2019. The lesions with clinical suspicion of lymphoma were excised and processed for histopathological examination. Depending on the histopathological features, a series of IHC markers was applied. The study included cases that were diagnosed as lymphoma after correlating histopathology and IHC findings. The lymphomas were categorised according to the 2017 WHO classification of tumours of hematopoietic and lymphoid tissue. **Results:** Hodgkin lymphoma (HL) constituted 23.9% (n 32) and non-Hodgkin lymphoma (NHL) constituted 76.1% (n 102) of the total lymphomas. DLBCL was the most common NHL and classic Hodgkin lymphoma was the most common HL. Cervical lymph node was the most common site of lymphoma and gastrointestinal lymphoma was the most common extranodal lymphoma. **Conclusion:** We came across more NHLs than HLs. The frequency of HL at our institute was more than other Asian countries but lesser than the western world. The number of T cell lymphomas that we received was less than that of south India.

KEYWORDS : Lymphoma, Immunohistochemistry, Hodgkin Lymphoma, Non Hodgkin Lymphoma

INTRODUCTION

Lymphomas are a diverse group of malignancies arising from B and T Lymphocytes and are categorized as either Hodgkin Lymphoma (HL) or Non Hodgkin Lymphoma (NHL). There is abundant immunologic and karyotypic evidence indicating these neoplasms contain clonal expansions of a single functional subpopulation. HL is an entity which was first described by Thomas Hodgkin in 1832 and is characterized by the presence of classic Reed-Sternberg cells and its variants in an appropriate cellular background. It is broadly classified into classic Hodgkin lymphoma (CHL) and nodular lymphocyte predominant Hodgkin Lymphoma (NLPHL). NHLs are a heterogenous group of lymphoproliferative disorders which originates from the different cells of the lymphoid system (B, T or Natural Killer Cells). Lymphomas constitute 3.69% of all Malignancies worldwide.

There is sparse literature regarding lymphoma from eastern India.[1-7] Our study aimed to analyse the subtype distribution of lymphomas on the basis of histopathology and immunohistochemistry in a single tertiary care centre from Eastern India.

MATERIALS AND METHODS

This was a retrospective, observational study comprising 134 cases of lymphoma over the last three years from August 2016 to July 2019. Detailed history was obtained and informed consent was taken. The lesions with clinical suspicion of lymphoma were excised. After the specimen was received in our department, it was processed for histopathological examination. On haematoxylin and eosin stained sections, the initial diagnosis of lymphoma was made. Depending on the histopathological findings, a panel of IHC markers was applied. Standard operating protocol was followed for IHC. The various IHC markers of lymphoma used were CD 19, CD 20, CD 22, CD 23, CD 79a, CD 3, CD4, CD 5, CD8, CD 10, CD15, CD30, CD 56, CD 68, CD138, BCL6, BCL2, Cyclin D1, MUM1, TdT and Ki67. The immunohistochemical findings were correlated with the histopathological features, to determine the lymphoma subtype. Our study included cases that were diagnosed as lymphoma after correlating histopathology and IHC findings. The cases where the IHC findings did not support the histopathological diagnosis of lymphoma, were excluded from the study. The lymphomas were categorised according to the 2017 WHO classification of tumours of hematopoietic and lymphoid tissue.

RESULTS

One thirty-four patients were included in the study. Most of the patients (n 95, 70.9%) belonged to the age group of 16-60 years, with >=61 years being the next largest group (n 31, 23.1%), followed by 0-15 years (n 8, 5.9%). The male to female ratio was 3.3:1. 69.4 % (n 93) lymphomas were nodal whereas 30.6% (n 41) lymphomas were extranodal. Among nodal lymphomas the most common site was cervical lymph node (70.1%) followed by inguinal lymph node (14.1%) and axillary lymph node (8.6 %). Other lymph nodal sites (7.2%) included internal iliac lymph node, mediastinal lymph node and retroperitoneal lymph node. Gastrointestinal lymphomas (n 11) were the most common extranodal lymphomas (n 41). Gastrointestinal lymphomas included lymphomas of stomach (n 6), duodenum (n 1), ileum (n 2), caecum (n 1) and colon (n 1). The other sites of extranodal lymphomas were tonsil, chest wall, urinary bladder, lacrimal gland, orbit, breast, gingivobuccal sulcus, gluteal region, maxilla, mandible, lung, mediastinum, nasal cavity, skin, soft palate, spleen and testis.

Hodgkin lymphoma (HL) constituted 23.9% (n 32) and non-Hodgkin lymphomas (NHLs) constituted 76.1% (n 102) of the total lymphomas. Among the HLs, classical HL (CHL) constituted 62.5% (n 20) and nodular lymphocyte predominant HL (NLPHL) constituted 37.5% (n 12). Nodular sclerosis was the most common CHL with 12 cases followed by mixed cellularity (8 cases). [Figure 1, a-d] [Table 1]. All HL subtypes were seen predominantly in males. The mean age for NLPHL was 36.7 years (range 20-61) and the mean age for CHL was 36.4 years (range 6-70).

Table 1: Subtype and age distribution of the total Hls

Subtype of HL	Number of Cases (n=32)	Age range & Mean age
Classical HL	16 (50%)	6-70 years, 36.4 years
NLPHL	10 (31.2%)	20-61 years, 36.7 years
Unclassifiable HL	06 (18.8%)	-

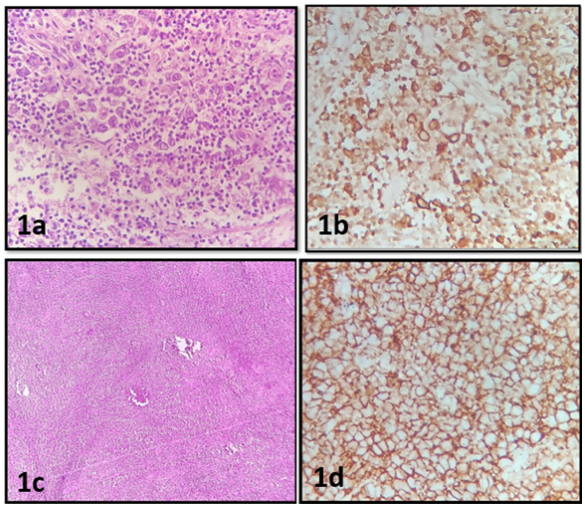

Figure 1 a-d : 1a) Classical HL- Section shows classic Reed Sternberg cells and Hodgkin cells in a mixed inflammatory background (H&E, X400). 1b) Classical HL - CD 30 positivity in Reed Sternberg cells and Hodgkin cells (IHC). 1c) NLPHL- Section shows replacement of nodal architecture by expansive vague nodules of lymphocytes (H&E, X100). 1d) NLPHL- CD 20 positivity in neoplastic cells. (IHC)

Table 2 shows the subtype distribution of the NHLs. Of all the NHLs (n 102), B cell NHL constituted 90.2 % (n 92) and T cell NHL constituted 9.9% (n 10). The most common NHL was diffuse large B cell lymphoma (DLBCL) (n 44, 43.1%) followed by follicular lymphoma (FL) (n 12, 11.7%) [Figure 2]. Other NHL subtypes included B cell acute lymphoblastic lymphoma (B ALL) (n 6, 5.7%), small lymphocytic lymphoma (SLL) (n 6, 5.9%), extranodal marginal zone lymphoma of mucosa associated lymphoid tissue (MALT lymphoma) (n 8, 7.7%), mantle cell lymphoma (MCL) (n 8, 7.7%), marginal zone lymphoma (MZL) (n 6, 5.9%) and Burkitt lymphoma (n 2, 1.8%). DLBCL was the most common gastrointestinal lymphoma, followed by MALT lymphoma.

Table 2: Subtype distribution of the total NHLs

Subtype of NHL	Number of cases (n=102)
DLBCL	44 (43.1%)
Follicular Lymphoma	12 (11.7%)
B ALL	06 (5.7%)
SLL	06 (5.9%)
Malt Lymphoma	08 (7.7%)
MCL	08 (7.7%)
MZL	06 (5.9%)
Burkitt Lymphoma	02 (1.8%)
T cell lymphoma	10 9.9%)

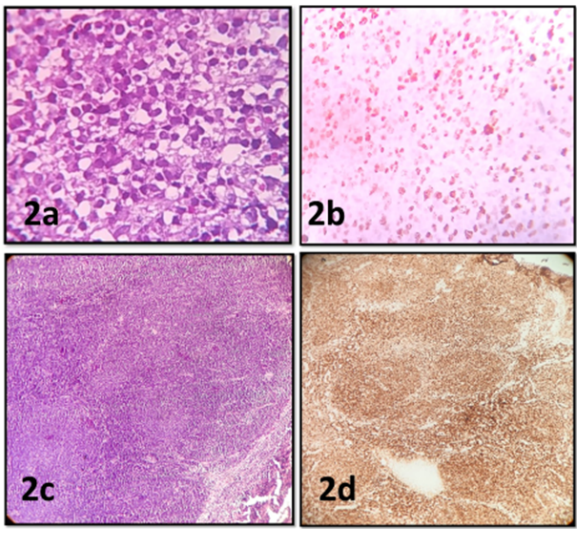

Figure 2a-d: 2a) DLBCL- Section shows diffuse growth pattern with large cells (H&E, X400). 2b) DLBCL- Ki 67 LI is 50%. (IHC) 2c) Follicular lymphoma- Section shows closely packed poorly defined neoplastic follicles with attenuated/ absent mantle zone. (H&E, X100). 2d) Follicular lymphpma- The neoplastic follicles are strongly positive for BCL2. (IHC)

Among 10 cases of T cell NHL, there were 5 cases of peripheral T cell lymphoma, not otherwise specified, 2 cases of angioimmunoblastic T cell lymphoma and 1 case of anaplastic lymphoma. 2 cases of mature T cell NHL could not be further categorised.

DISCUSSION

The incidence of lymphomas has increased over years which can be attributed to genetics, drugs, immunosuppression and chemicals.[8-10] The geographic distribution of lymphoma varies and it is influenced by genetic and environmental factors. The study of the distribution pattern of lymphoma will facilitate in better understanding of its predisposing factors.

In our study, adults were more frequently involved than the paediatric population. The overall male to female ratio was 3.3:1. The most common site of lymphoma was cervical lymph node while the commonest site of extranodal lymphoma was gastrointestinal tract.

Our study showed 76.1% cases of NHL and 23.9% cases of HL which was similar to the findings by Arora et al in southern India where they reported 78.7% cases of NHL and 21.3% cases of HL. [1] The frequency of HL was lower in other Asian countries with the same being 7%, 8.5% and 6.6% in Japan, Thailand and China respectively. [11,12] The frequency of HL reported in the West was 31% which was more than that of ours. [13]

We reported 102 cases of NHL, out of which 90.2% comprised of B Cell lymphomas and 9.8% comprised of T Cell lymphomas. In the study by Arora et al in southern India, B cell lymphoma accounted for 78.6% and T cell/NK cell lymphoma accounted for 20.2 % of the total NHLs. [1]

The most common NHL was DLBCL in our study cohort. Globally, the most common lymphoma is DLBCL, representing approximately one-third of all cases. In the 2017 WHO classification of tumours of hematopoietic and lymphoid tissue, most cases of DLBCL have been designated as not otherwise specified (NOS) while about 20% of cases have been assigned specific variants of DLBCL. These variants, are specified on the basis of distinctive morphological or immunophenotypic features or distinctive clinicopathological features. [14] We have designated all our cases of DLBCL as NOS.

Gene expression profiling (GEP) methods have been used to study DLBCL. Alizadeh and colleagues27 were among the first to apply GEP methods to the study of DLBCL. They divided the cases of DLBCL according to cell-of-origin into germinal centre B cell like (GCB) and activated B-cell like (ABC) subtypes, as well as a small unclassifiable group.[15] Various IHC algorithms have been developed as surrogates for GEP to predict cell-of-origin, out of which, the Hans algorithm was the first reported and is most commonly used. [16] By applying this algorithm, 50 % of our DLBCLs were classified into GCB subtype showing CD 10+ or CD10-/BCL6+/MUM1- . 40%% of our DLBCLs were of ABC subtype showing CD 10-/MUM1+. 9% could not be classified into either types. Despite the limitations of IHC algorithm, we considered it to detect cell of origin of DLBCL as GEP method is not available at our institute.

Follicular lymphoma has been reported to be the second most common lymphoma diagnosed in the United States and Western Europe,1 representing approximately 35% of all NHL and 70% of indolent lymphomas.[17] In our study cohort the second commonest NHL was FL. Amongst T cell lymphomas, Peripheral T cell lymphoma, not otherwise specified was the commonest.

In our study, the gastrointestinal lymphomas were the most common extranodal lymphomas. DLBCL was the most common gastrointestinal lymphoma followed by MALT lymphoma. Similar results were found in studies by Bautista-Quach et al and Olszewska-Szopa et al.[18,19] T- and NK-cell NHL of the gastrointestinal tract are uncommon, and we did not come across such a case but they are important to recognise as there may be morphological and immunophenotypic overlap between lymphoid lesions with vastly different clinical outcomes.

We encountered several pitfalls while diagnosing lymphomas. In a few cases the initial diagnosis made on histopathology was rectified after corelating the histopathological features with immunohistochemical findings. Table3 shows the list of misdiagnosed lesions on histopathology. In tonsil a case of DLBCL was wrongly diagnosed as poorly differentiated carcinoma. On IHC, positivity for CD19, CD20, CD79a and BCl6, Ki 67 labelling index of 60 % and negative staining for CK 5/6 and p63 favoured diagnosis of DLBCL. A case of extranodal marginal zone lymphoma of mucosa associated lymphoid tissue (MALT lymphoma) of tonsil was misdiagnosed as poorly differentiated carcinoma. As this tumour was positive for B cell markers and negative for CK 5/6, p63, CD5, BCL6 and CD10, the final diagnosis of MALT lymphoma was made. MALT lymphoma of tonsil is rare with sparse literature describing the same.[20]

A nasal cavity tumour was initially diagnosed as poorly differentiated carcinoma due to presence of sheets of large atypical cells but on IHC the tumour cells were positive for B cell markers, CD 10 and BCL6. As a result, it was ultimately diagnosed as DLBCL. NHLs of the nasal cavity are uncommon representing 3% to 5% of all malignancies. Early diagnosis is essential for proper treatment, and lymphomas must always be included in the differential diagnosis of lesions of the nasal cavity.[21] In our study a DLBCL of axillary lymph node was initially misdiagnosed as metastatic carcinoma.

In duodenum a tumour initially diagnosed as gastrointestinal stromal tumour showed negative staining for CD34 and CD117 while immunostains for B cell markers, CD10 and BCL6 were positive, thereby leading to final diagnosis of DLBCL. Similarly, in a case report by Suh BJ, a case of malignant gastrointestinal stromal tumour was initially misdiagnosed as malignant B-cell lymphoma.[22] In our study, a DLBCL of stomach was misdiagnosed as poorly differentiated carcinoma which was rectified after IHC studies.

Table 3 List of misdiagnosed lesions on histopathology

SN.	Site	Histopathological diagnosis	Final diagnosis after corroborating histopathological and IHC features
1	Tonsil	Poorly differentiated carcinoma	DLBCL
2	Tonsil	Poorly differentiated carcinoma	MALT lymphoma
3	Nasal cavity	Poorly differentiated carcinoma	DLBCL
4	Axillary lymph node	Metastatic carcinoma	DLBCL
5	Duodenum	Gastrointestinal stromal tumour	DLBCL
6	Stomach	Poorly differentiated carcinoma	DLBCL
7	Lymph nodes	Lymphoma	Reactive lymphadenitis

Four lymph node swellings were initially diagnosed as lymphoma on histopathology but on IHC the staining pattern of CD20, CD3, BCL6 and CD10 favoured diagnosis of reactive lymph node. This highlights the fact that in certain cases histopathology alone is not sufficient to differentiate lymphomas from reactive lymph node. The aforementioned four lesions were excluded from our study.

CONCLUSION

In this study we analysed the subtype distribution of lymphomas on the basis of histopathology and immunohistochemistry and elucidated the pitfalls in histopathological diagnosis of lymphoma. We came across more NHLs than HLs. The frequency of HL at our institute was more than other Asian countries but lesser than the western world. DLBCL was the most common NHL and CHL was the most common HL. The number of T cell lymphomas that we received was less than that of south India. Cervical lymph node was the most common site of lymphoma and gastrointestinal lymphoma was the most common extranodal lymphoma.

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