A morphological study with clinical, pathological, and immunohistochemical characterization of Non-Hodgkin Lymphoma

Running title: Morphological Study of Non-Hodgkin Lymphoma

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Abstract

Background: Non-Hodgkin lymphoma (NHL) represents a heterogeneous group of lymphoproliferative malignancies with unique presentation and treatment response. This study was intended to assess the histomorphology of non-Hodgkin lymphoma subtypes and correlate with clinicopathological and immunohistochemical findings.

Methods: This retrospective study was conducted on all specimens diagnosed as NHL by histopathological analysis, with immunohistochemical correlation in the pathology department, for two years. Clinical details such as age, gender, site of the lesion, and Nodal / extranodal presentation were recorded. Histopathological analysis was performed, and Immunohistochemical (IHC) reports were obtained. Further histopathological findings were correlated with IHC results. Statistical analysis was done based on the frequency distribution.

Result: This study includes 48 cases. Most cases are 61 to 70 years old,with a male predominance (56.25 %). The most common clinical diagnosis was lymphoma (79.17 %). Most of the lesions were of lymph nodal origin (60.42 %). The cervical group (35.42 %) is the most common lymph node affected, followed by the axillary nodes. The most common extranodal sites are the retroperitoneum and stomach. On histopathological evaluation, the most common diagnosis was NHL (68.75 %) without further subtyping. Among cases where subtyping was done, the most common lesion were follicular lymphoma and diffuse large B-cell lymphoma (DLBCL). On IHC evaluation, B cell neoplasms (85.42%) were common compared to T cell neoplasms (14.58 %). The most common subtype was DLBCL (52.08 %), followed by follicular lymphoma (16.67 %).

Conclusion: Our study found diffuse large B cell lymphoma (DLBCL) as the most common type of Non-Hodgkin's Lymphoma. Cervical lymph nodes were found to be the most common site of involvement. But the involvement of rare sites like the testis and palate was also found. Hence the probability of NHL in these rare sites should always be considered.

Keywords: Non-Hodgkin's Lymphoma, Immunohistochemistry, Histomorphology

Introduction

Lymphadenopathy is a frequent clinical problem, and biopsies are often used to establish the cause of enlarged lymph nodes. It may be neoplastic or non-neoplastic. The neoplastic conditions are mainly metastases and lymphohematogenous malignancies. The non-neoplastic lymphadenopathy has a diverse etiology and can be caused by infections, drug reactions, and non-neoplastic lymphoproliferative disorders like Castleman disease (1).

Numerous classifications of NHL have been proposed, starting with the first one presented by Gall and Mallory in the early 19th century. In 1982, a working formula (WF) was proposed. This working formula divided NHLs into three histological groups depending on the clinical behavior: low, moderate, and high grade. REAL (Revised European American Lymphoma) classification was proposed in 1994, taking into account various shortcomings of WF. The REAL classification is based on morphology, immunophenotypic and cytogenetic data in lymphoma cases (2).

It is the 11th most common cancer worldwide, with a five-year survival rate of 35 percent.

In terms of incidence, the disease accounts for approximately 5.1% of all cancer cases and 2.7% of all cancer deaths. In India, incidence rates for NHL in men and women are 2.9/100,000 and 1.5/100,000, respectively (3). In India, there are approximately 23,718 new NHL cases reported each year (4).

NHLs are slightly more frequent in developed countries (50.5 % of cases worldwide), with the highest rates in Australia and North America and relatively low rates throughout Asia and Eastern Europe (5). In Asia, the incidence of NHL has increased in recent years. Within India, the incidence is several-fold higher in the urban population compared to the rural population. Hence, urban lifestyles and economic progress may increase cancer incidence (3).

The diagnosis of NHL is usually made on Hematoxylin and Eosin-based morphological examination. Immunohistochemistry can differentiate the lymphocytes into T and B cells (6). Various specimens like lymph nodes, bone marrow aspirates, trephine biopsy cores, peripheral blood, and other fluids such as cerebrospinal fluid (CSF), ascitic fluid, and pleural aspirates can be used for lymphoma diagnosis, depending on the presenting clinical symptoms (3).

Non-Hodgkin lymphoma (NHL) comprises a heterogeneous group of neoplasms arising from different lymphoid cell lineages at various stages of development. Non-Hodgkin Lymphomas have discrete causes and demonstrate unique patterns of behavior and responses to the treatment given (7). It is characterized by the neoplastic proliferation of lymphoid tissue, including lymphocytes, histiocytes, and their precursors (3).

NHL etiology is mainly unknown. Infectious agents, severe immunodeficiency, blood transfusion, pesticides, and solvent contact have been constantly reported with increased NHL risk (8). It generally presents as painless, localized, or generalized lymphadenopathy (9). It may present as an extranodal lesion, too. The gastrointestinal tract is the most common extranodal site for NHL, and the other areas include the testis, bones, eyes, brain, heart, white blood cells, skin, and kidneys (10).

NHL can be subclassified based on the stage of maturation into the immature and mature types of NHL. On the other hand, it can be subclassified based on the cell of origin into B cell, T cell, or natural killer cell [NK cell] type of lymphoma (11).

Immunohistochemistry (IHC) is applied in 3 circumstances: It determines the phenotype of the abnormal population detected by morphology, differentiates an abnormal population recognized by flow cytometry, and screens apparently ‘‘reactive’’ tissue to determine whether a subtle abnormal population is present (11).

The occurrence of NHL is higher in the male population in India and worldwide (4,1). NHL in India has a median age of 54 years, a higher male-to-female ratio, a higher proportion of patients with B-symptoms, higher frequency of diffuse large B-cell lymphomas (11).

This study is conducted to assess the clinical presentation of various NHL subtypes, taking IHC as the gold standard, and to study the histo-morphological subtypes of NHL with immunohistochemical correlation.

Methods

This retrospective study was conducted on all specimens diagnosed as NHL by immunohistochemical staining in the pathology department, Father Muller Medical College, Mangalore, Karnataka, India, for 24 months, from March 2018 to February 2020. Institutional ethical clearance was obtained (FMrEC/CCM /s3e/2020). The specimens were fixed in 10 % formalin, and representative sections were obtained. Clinical details such as age, gender, site of the lesion, and Nodal / extranodal presentation are recorded. Histopathological analysis was performed, and Immunohistochemical reports were obtained. Further histopathological findings were correlated with IHC results. Statistical analysis was done based on frequency.

Result

This study includes a total of 48 cases. The majority of the cases are in the age range of 61 to 70 years (14/48 = 29.17%), followed by 51 to 60 years (9/48 = 18.75%). Five cases (10.42%) were noted below 20 years. A male predominance (27/48= 56.25 %) is indicated in the study.
The most common clinical diagnosis was lymphoma (38/48= 79.17 %). Retroperitoneal malignancies were suspected in four cases (8.33 %). This study also included clinical diagnosis of Carcinoma Stomach, epidural abscess, gastrointestinal stromal tumor, grem cell neoplasm testis, neuroendocrine tumor of the small intestine, reactive lymphadenitis amounting to 1 case each (2.08 %).
The majority of the lesions were of lymph nodal origin (29/48 =60.42 %). The most common group of lymph nodes affected is the cervical group (17/48 = 35.42 %), followed by the axillary group (7/48 = 14.58%) and the inguinal group (2/48 = 4.17 %). The most common extranodal sites are the retroperitoneum (5/48 = 10.42%) and stomach (4/48 = 8.33 %). The frequency of lesional sites is shown in Table 1.
On histopathological evaluation, the most common diagnosis was non-Hodgkin lymphoma (33/48= 68.75 %) without further subtyping. Among cases where subtyping was done based on histomorphology, the most common lesion was follicular lymphoma (3/48= 6.25%) and diffuse large B cell lymphoma (DLBCL) (3/48 = 6.25 %). The frequency of diagnosis is shown in Table 2. Histopathological pictures of DLBCL and Follicular lymphoma are given in Figure 1 and Figure 2, respectively.
On immunohistopathological evaluation, B cell neoplasms (41/48= 85.42%) were common compared to T cell neoplasms (7/48 = 14.58 %). The most common subtype of non-Hodgkin lymphoma was DLBCL (25/48 = 52.08 %), followed by follicular lymphoma (8/48 = 16.67 %). Other B-cell neoplasms observed in the study are mantle cell lymphoma, Burkitt lymphoma, high-grade B-cell lymphoma, marginal zone lymphoma, mediastinal large B-cell lymphoma, and plasmablastic lymphoma. T cell neoplasms observed in the study are angioimmunoblastic T cell lymphoma, adult T cell lymphoma, peripheral T cell lymphoma, precursor T lymphoblastic lymphoma, and anaplastic large cell lymphoma. The frequency of neoplasms is depicted in Table 2.
On comparing the clinical diagnosis and histopathological findings, 45 out of 48 cases were found to have correlating results. A clinically suspected case of the gastrointestinal stromal tumor was histopathologically diagnosed as DLBCL. Similarly, a case of a neuroendocrine tumor of the small intestine and epidural abscess was histopathologically diagnosed as DLBCL and peripheral neuroendocrine tumor (PNET)/ lymphoma, respectively. Further immunohistochemistry showed positivity for DLBCL markers in a clinically suspected and histopathologically diagnosed reactive lymphadenopathy case.

Discussion

NHL is found to be more common in developed countries. The highest incidence is noted in North America and Australia. NHL incidences are found to be low in South-Central and Eastern Asia.

NHL incidence is found to be higher in men. The worldwide age-standardized rate (ASR) is 6.1/100,000 compared to women (ASR 4.2/100,000). In the present study, males (56%) are also more than females (44%). In studies conducted by Kalyan et al. and Morton et al., they also found that the incidence of NHL is higher in the Male gender (12,13).

NHL incidences are more according to various studies, NHL incidences are more in older age groups, and the highest incidence is in the 5^{th} and 6^{th}decades of life.

In a study conducted by Roy et al., they concluded that 63.6% of NHL incidence is more common in the age group of 51 -60 (1). Similarly, in a study, Vallabhajosyula et al. concluded that the median age of the study population was 55.5 years (14). Padhi et al. also reported that the highest incidence was during the 4th to 5th decade of life (15).

In a single-center study conducted on seventy-seven cases by Sharma et al. to determine the distribution of the significant subtypes of non-Hodgkin lymphoma, they concluded that B-cell lymphomas constitute 89.3%. On the other hand, T-cell lymphomas formed 10.7% of the NHLs. They found Diffuse Large B-Cell Lymphoma (DLBCL) as the most common subtype of NHL, constituting 46.8% of all NHLs. Follicular Lymphoma (FL) and MCL are less common in India compared to Europe and the US (7). In the present study, DLBCL is also noted to be the most common type of NHL. The findings in various studies are compared in Table 6.

In a study by Gupta et al. Including a hundred cases for immunohistochemical analysis with clinicopathological correlation in NHL, they concluded that NHL subtypes in India have differences in distribution compared to the rest of the world (3). They concluded that PAX 5 could be used as a universal single pan B cell marker, whereas CD5 is a pan T cell marker in a resource-poor setting by Borgohain et al. on 50 patients to determine the diagnostic Utility of Immunohistochemistry in Lymphoma concluded that lymphoma incidences have been showing a worldwide increase in recent years. Non-Hodgkin lymphomas are more common compared to Hodgkin's lymphomas. Diffuse large B cell lymphomas are found to be more common in India; in contrast, follicular lymphoma and Mantle cell lymphomas are less common compared to Europe and the U.S.A. Histopathology and immunohistochemistry play a vital role in diagnosing lymphoma and subtyping (16).

In a study conducted by Aggarwal et al., including 52 NHL cases, they concluded that even though REAL classification requires a detailed IHC panel, the use of a basic panel for B- and T-cell markers allows the distinction between B- and T-cell lymphomas. Therefore, REAL classification should be employed for NHL categorization even in smaller centers with limited IHC panels (17). They found DLBCL as the most common NHL subtype. In various studies conducted by Naresh et al., El-Esawy et al., and Nair et al., they also concluded that DLBCL is the most common subtype (18,19,20).

In a study conducted in Pakistan by Mushtaq et al., they also observed DLBCL as the most common subtype (21).

Conclusion

Non-Hodgkin lymphoma incidences are increasing worldwide. The Indian population is also noted to have high incidences of NHL. DLBCL and follicular lymphoma are the most common NHLs in the current study. Immunohistochemistry helps subclassify NHL and differentiate reactive tissue from malignancy. Cervical lymph nodes were the most common site of involvement. But rare places like the testis and parasacral areas were also noted in the study.

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Conflict of interest
The authors declare that they have no competing interests

Author's contributions
JP helped with IHC interpretations. All authors read and approved the final manuscript.

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Table 1. Distribution frequency of Lesion site

Lesional site	Frequency	Percentage
Cervical Lymph Node	17	35.42
Axillary LN	7	14.58
Retroperitoneal mass	5	10.42
Stomach	4	8.33
Abdominal mass	3	6.25
mediastinal mass	3	6.25
Inguinal LN	2	4.17
Hard Palate	1	2.08
Mesenteric lymph node	1	2.08
Pleura	1	2.08
Small intestine	1	2.08
Spleen	1	2.08
Testis	1	2.08
Tonsil	1	2.08
Total	48	100.00

Table 2. Histopathological diagnosis and immunohistochemistry diagnosis

Histopathological diagnosis	Frequency	Percentage	IHC Diagnosis	Frequency	percentage
Non-Hodgkin Lymphoma	33	68.75	DLBCL	25	52.08
Diffuse large B cell lymphoma	3	6.25	Follicular lymphoma	8	16.67
Poorly differentiated carcinoma/ Lymphoma	3	6.25	Angioimmunoblastic T-cell lymphoma	3	6.25
Follicular lymphoma	3	6.25	Mantle cell lymphoma	3	6.25
Lymphoma/ Germ cell neoplasm	1	2.08	Adult T cell lymphoma	1	2.08
MALT lymphoma	1	2.08	Anaplastic large cell lymphoma	1	2.08
Plasmablastic lymphoma	1	2.08	Burkitt lymphoma	1	2.08
PNET/Lymphoma	1	2.08	High-grade B cell lymphoma	1	2.08
Poorly differentiated carcinoma	1	2.08	Marginal zone lymphoma	1	2.08
Reactive lymphadenitis	1	2.08	Mediastinal large B-cell lymphoma	1	2.08
Total	48	100.00	Peripheral T-cell lymphoma	1	2.08
			Plasmablastic lymphoma	1	2.08
			Precursor T Lymphoblastic lymphoma	1	2.08
			Total	48	100.00

Table 3. Comparison with various other studies

Studies on NHL	Most common NHL	2nd most common	3rd most common
Gupta et al. (3)	DLBCL	Peripheral B-cell NHL -NOS	Peripheral T-cell lymphoma
Akhter et al. (10)	DLBCL	SLL/CLL	Peripheral T-cell lymphoma
Sharma et al. (7)	DLBCL	SLL/CLL	Mantle cell lymphoma
Present study	DLBCL	Follicular lymphoma	SLL/CLL

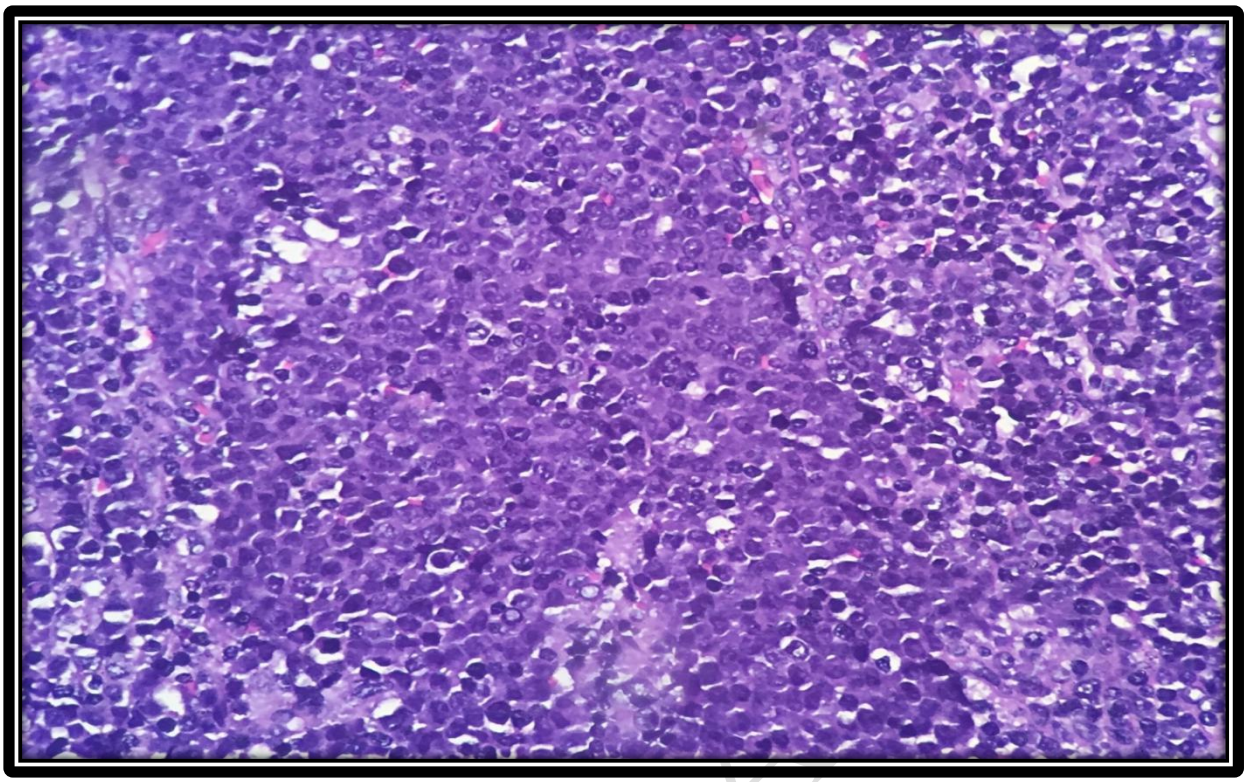

Figure 1. Diffuse large B cell lymphoma (H&E, 400x)

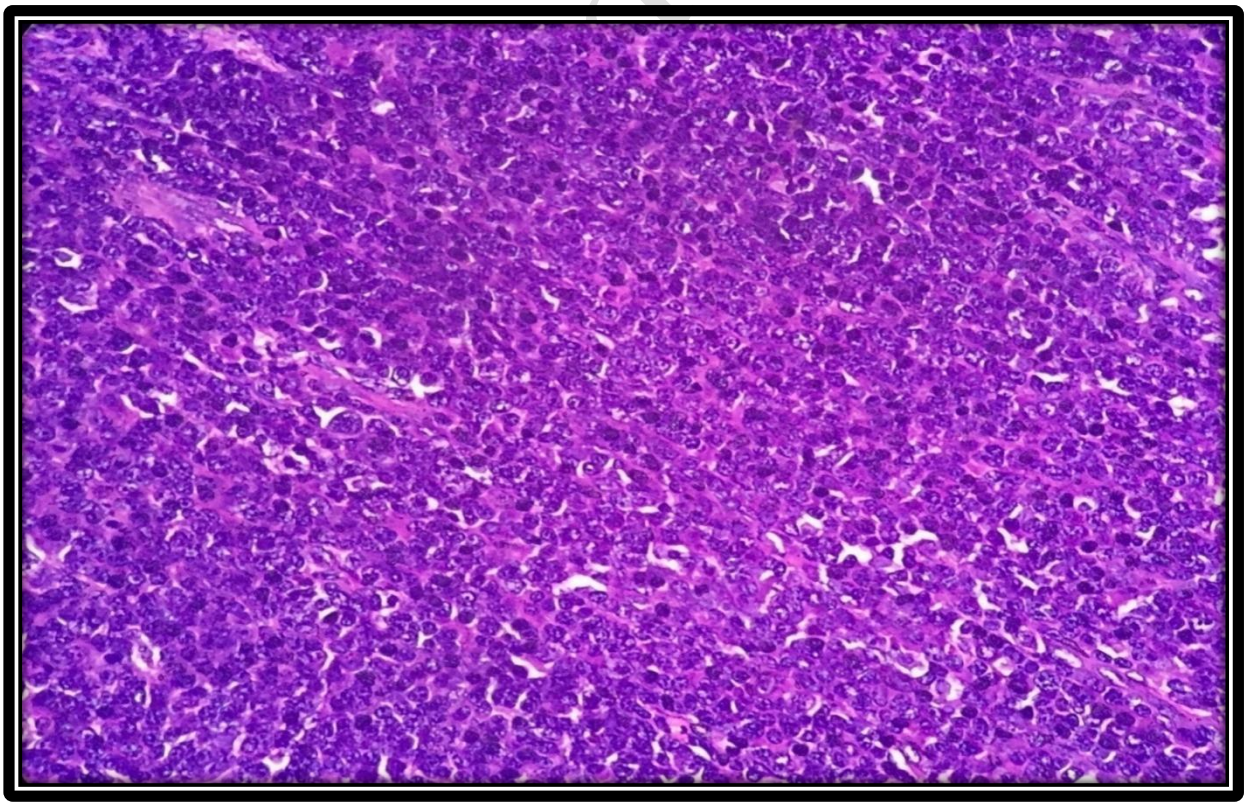

Figure 2. Follicular Lymphoma (H&E ,400x)