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Comparison of immunohistochemistry with conventional histopathology for evaluation of lymphnodes

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Abstract

Background: Lymph node specimens are routinely used for the evaluation of lymphadenopathies. Tuberculosis and reactive lymphadenitis are the major causes of lymphadenopathy in developing countries. A panel for Immunohistochemistry is decided based on differential diagnosis through histopathology. Immunohistochemistry helps in the sub typing of the lymphomas and metastatic lesions.

Materials and Methods: The present study was carried out on 50 patients. Types of samples include lymph node specimens received in histopathology section of the pathology department. The cases were diagnosed based on light microscopy and confirmed by Immunohistochemistry.

Aims and Objectives:

- To determine the histopathological spectrum of lymphadenopathy by evaluation of the specimen.
- To study the incidence of lymph node lesions with respect to age and sex.
- To use Immunohistochemistry for subtyping of various neoplastic lesions.

Results: A total of 50 lymph node specimens were studied. Age distribution varied from 7 to 80 years with female preponderance. Non – Neoplastic lesions were common comprising of 27 cases (54%) while neoplastic lesions were 23 (46%). Among non neoplastic lesions reactive lymphadenitis (26%) was common followed by tuberculous lymphadenitis (20%). In neoplastic lesions, metastatic diseases (20%) predominated.

Conclusion: Reactive lymphadenitis and tuberculosis are the most common diagnosis in lymph node specimens. Histopathology with IHC on lymph node specimens are diagnostic and reliable investigations to differentiate non-neoplastic lesions from neoplastic lesions, and further classify the disease in both cases.

Keywords: Non neoplastic, neoplastic, lymphnode, Immunohistochemistry

Introduction

Scattered throughout the body, lymph nodes perform the critical function of filtering both exogenous and endogenous antigens and producing immune response through activated lymphocytes and antibodies. They can become enlarged in infections, autoimmune diseases, lymphomas, and metastatic carcinomas ^[1].

Lymphadenopathy is a common clinical problem and biopsies are usually undertaken to determine the cause of nodal enlargement, which may be neoplastic or non-neoplastic ^[2]. Lymph node lesions form a wide range of spectrum from benign reactive changes to lymphoma and metastatic deposits ^[3]. Lymphadenopathy is either generalized or localized, Generalized lymphadenopathy is seen in a large number of systemic illnesses while localized lymphadenopathy is more often seen with local infection or malignancy ^[4]. First step in developing better therapies is the recognition of distinct specific disease entities by pathologists. Since there is no specific treatment for most forms of reactive lymphadenopathy, even a non-specific diagnosis is helpful, because the main aim is to exclude a malignant process and treatable causes ^[4].

Immunohistochemistry helps in the sub typing of the lymphomas into different categories which have a therapeutic and prognostic importance ^[5]. Metastasis in neck lymph nodes from squamous cell carcinoma is a common situation in cases of patients already diagnosed and treated for cancer of the head and neck, and it may as well be the first overt clinical manifestation.

In general, squamous cell carcinoma, even the metastatic one, does not pose major histopathological diagnostic difficulties; however, in their less differentiated forms, it may raise diagnostic questions when only hematoxylin and eosin dyes are utilized.

Immunohistochemistry brought about a diagnostic and prognostic revolution in the evaluation of primary neoplasms of the hematopoietic system. The classification and identification of lymphomas was positively impacted, and we started to add the method to daily diagnostic routine, which is presently a mandatory item in pathology reports.

This study is to evaluate the incidence with respect to age and gender along with histopathological patterns of lymph nodes received in our department over a period of one year.

Aim and objectives

- To determine the histopathological spectrum of lymphadenopathy by evaluation of the specimen.
- To study the incidence of lymphnode lesions with respect to age and sex.
- To use Immunohistochemistry for subtyping of various neoplastic lesions.

Type of study: Prospective & Retrospective study.

Materials & methods

- Place of study: The present study was carried at the department of pathology in Histopathology & IHC section of our hospital.
- Design of study: A diagnostic prospective & retrospective study.
- Duration of study: 1 year
- **Sample size:** Total 50 cases.
- **Sample types**:-Lymphnode specimens received in histopathology section of the pathology department.

Clinical work up, radiological investigations and detailed history of present complaint and local examination were taken from requisition forms in histopathology section.

For resected specimen & biopsy

The representative areas from the specimen and biopsies were submitted for histopathological examinations. Tumour tissue blocks of 2cm x 2cm x 0.5cm was collected and fixed in 10% neutral buffered formalin solution for 24 hours and subsequently subjected to histological processing and paraffin embedding. Histological paraffin sections, 3-5um thick, were taken. H & E staining was performed and examined by light microscopy. The diagnosis was given according to morphology.

IHC panel

IHC using relevant antibodies was done according to histomorphological features needed. wherever Immunohistochemical studies were carried out with 3-5u paraffin sections. The antibodies included CD3, CD 5, CD15, CD20, CD 23, CD30, PAX 5, Bcl-2, Bcl-6, cyclin D1 and Ki67. The lymphomas were classified according to World Health Organization classification of hematolymphoid malignancies 2022. All cases of non Hodgkin lymphomas were subjected to B and T cell markers for sub typing. Likewise most of the Hodgkin lymphomas were subjected to immunophenotyping using CD 45, CD 15 and CD 30. In cases of ambiguity Pancytokeratin, P63, ER and PR were also used to exclude metastatic deposits.

Inclusion criteria

Biopsies and whole specimen of lymphnodes were included in the study.

Exclusion criteria

Case with inconclusive diagnosis due to lack of adequate material will be excluded.

Result

This study included a total of 50 lymph node specimens. The age range was 7-80 years and maximum cases were seen in the 21-40 years age group. There was female preponderance (56%) and male to female ratio was 1:1.27 The common site was cervical lymphadenopathy seen in 65% of cases followed by axillary (10%), inguinal (12%), supraclavicular (3%), mediastinal (4%), retroperitoneal (2%) and other sites (4%). In the 50 lymph node biopsies analyzed, the non neoplastic lesions were common comprising 54% (27 cases) and neoplastic lesions were 46% (23 cases).

In non neoplastic lesions, reactive lymphadenitis was the most common accounting to 26 % (13 cases) (Figure 1A). In these 13 cases, chronic non specific lymphadenitis (17%) was common followed by follicular hyperplasia (6%) and sinus histiocytosis (3%). Granulomatous lymphadenitis was the second common non-neoplastic lesion comprising 20% (10 cases) (Figure 1B) was the most common (Table 1). Non neoplastic lesions also included 1 case of Angiolymphoid Hyperplasia with eosinophilia (Figure 2), 1 case of Lymphangioma, 1 case of schwannoma and 1 case of lymphoepithelial cyst (Table 1)

In the 23 neoplastic lesions, metastatic diseases were common accounting to 20% (10 cases) followed by 16% (8 cases) of Non Hodgkin lymphomas and 10% (5 cases) of Hodgkin lymphomas (Table 2). In these neoplastic lesions 7 cases had a differential diagnosis of lymphoma/ metastatic lesion on histopathology which were later confirmed with the aid of Immunohistochemical markers CD 45 and Pancytokeratin, of which 4 cases were diagnosed as metastatic lesion and 3 cases were non Hodgkin lymphoma. In metatstaic lesions, Squamous cell carcinoma (5 cases) was the most common diagnosis followed by 2 cases of ductal carcinoma of breast (Figure 5), 2 cases of Adenocarcinoma, 1 case of thyroid malignancy. In the 8 cases of non Hodgkin lymphoma, 3 cases were of diffuse large cell lymphoma (Figure 4), 2 cases of small cell lymphocytic lymphoma, 1 case each of anaplastic large cell lymphoma, follicular lymphoma and T cell lymphoma. Immunohistochemistry was done in 23 cases in which 6 cases were positive for CD 20 (Figure 4B). 3 cases were positive for BCl2 (Figure 4C) and 2 cases were positive for CD 3. Hodgkin lymphomas include 5 cases, 4 cases were of classical type and 1 case was lymphocyte predominant type. In classical type of Hodgkin lymphoma, 3 cases were of mixed cellularity sub type (Figure 3), 1 cases of nodular sclerosis sub type. Classical Hodgkin lymphoma were confirmed by positivity for CD15 and CD30 (Figure 3B).

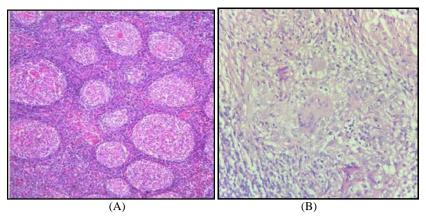


Fig 1: (A) Section shows histology of lymphnode showing follicular hyperplasia and sinus histocytosis (H & E 10x) (B) Section from axillary lymph node showing well-formed granuloma formation with Langhan's type of giant cell. (H & E 40x)

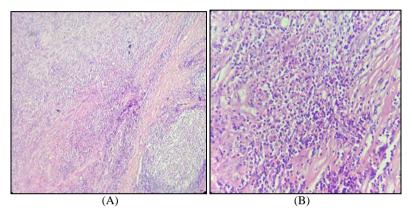


Fig 2: (A) Section from neck swelling showing lymphoid hyperplasia. (H & E 10x)(B) Vascular proliferation along with infiltration by eosinophils and lymphoid cells in a case of angiolymphoid hyperplasia with eosinophilia (H & E 40x)

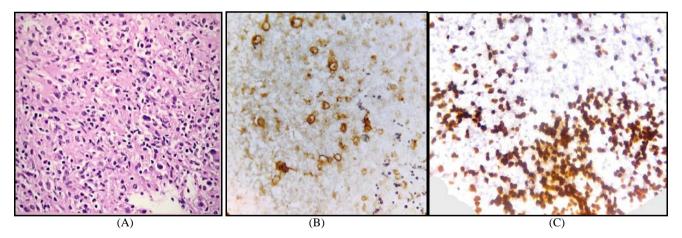


Fig 3: Mixed cellular subtype of Hodgkin's Lymphoma (A)Section from cervical lymphnode showing mononuclear RS cells(H & E 40x) (B)IHC marker CD 30 postive in RS cells (H & E 40x) (C)IHC marker PAX 5 weak positive in RS cells (H & E 40x)

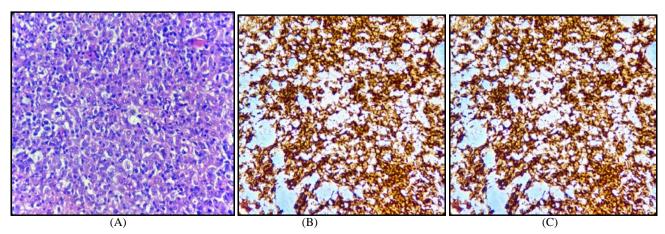


Fig 4: Diffuse Large Cell type of Non Hodgkin's Lymphoma (A) Section showing large cells with vesicular chromatin and prominent nucleoli. (B & C)IHC markers CD 20 positive and Bcl 2 positive in large cells (H & E 40x)

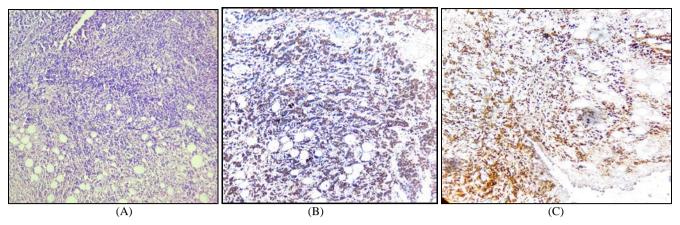


Fig 5: (A) Section shows metastasis from breast carcinoma showing malignant ductal epithelial cells (H & E 10x) (B & C) IHC markers Pan CK and ER positivity (H & E 10x)

Serial no Histopathological diagnosis Number of cases Reactive Lymphadenitis 2 Granulomatous lesion 10 3 Angiolymphoid hyperplasia with eosinophilia 1 4 Lymphangioma Schwannoma 5 6 Lymphoepithelial cyst Total:27

Table 1: Non neoplastic lesions of lymphnode (n=27)

Table 2: Neoplastic lesions of lymphnode (n=23)

Serial no	Type of lesion	Number of cases
1	Metatstatic lesions	10
2	Non Hodgkin's Lymphoma	8
3	Hodgkin's Lymphoma	5
		Total: 23

Table 3: Sex Distribution of lymphnode lesions (n=50)

Sex	Frequency	Percentage
Female	28	56%
Male	22	44%
Total	50	100%

Table 4: Age Distribution of lymphnode lesions (n=50)

Age (years)	Number of cases	Percentage
0-20	10	20%
21-40	21	42%
41-60	11	22%
61-80	8	16%
Total	50	100%

Discussion

Lymphadenopathy offers an important diagnostic clue to the etiology of the underlying condition. Though fine needle aspiration cytology is commonly used to establish the etiological diagnosis, excision biopsy of the lymph node remains the gold standard for diagnosis. This study included a total of 50 lymph node specimens with different histopathological patterns and Immunohistochemistry was done in lymphomas for confirmation and also to differentiate lymphoma from carcinoma.

Adeniji KA *et al.* ^[6] Adesuwa N *et al.* ^[7] and Roy A *et al.* ^[8] in their studies reported a male: female ratio of 1.6:1, 1.3:1 and 1.7:1 respectively indicating male preponderance, whereas, Mbata GC *et al.* ^[9] reported a male: female ratio of 1:1.3 showing female preponderance. In the present study, male: female ratio is (1:1.27) which is in concordance with

Mbata GC et al. [9] study.

In the present study, age ranged between 7 to 80 years. Maximum numbers of cases were seen in the age group 21-40 years (21 cases, 42%). The most common site for lymphadenopathy in our study was cervical (65%) followed by axillary nodes. Similar findings have been reported by number of different studies ^[7, 9, 10]. The preponderance of cervical lymphadenopathy may be related to its location near a common primary site of infections and malignancy that are drained through this single channel (the cervical lymph nodes) ^[11].

In the present study, benign lesions constituted a total of 27 (54%) cases. The most common benign diagnosis on histopathology was reactive lymphadenopathy in 13(26%) patients. Malignancies comprised 23 (46%) of cases, with lymphomas predominating accounting for 13 (26%) cases. Among the lymphomas, non-hodgkin's lymphomas (NHLs) were more common accounting for 8 (16%) cases of lymphadenopathies. Hodgkin's Lymphoma constituted 5 (10%) with mixed cellularity as the commonest form. Metastases constituted the remaining malignancies representing 10 (20%) cases of palpable enlarged peripheral nodes.

Lymphadenopathy is common in the first three decades of life; with reactive lymphadenopathy more common in the early years of life, Granulomatous lymphadenitis common in the young adult and malignancy seen more in the adult and elderly people. Analysis of lymphadenopathy in the clinical practice in the developing nations of the world has shown that infection remains an important cause with Tuberculosis as the most common aetiology in most areas. Nonspecific causes (reactive hyperplasia) and upper respiratory tract infections due to bacterial and viral agents are also important cause of lymphadenopathy in the developing world [9].

Metastatic deposits were common in neoplastic lesions accounting to 20%, followed by Non Hodgkin lymphomas (16%) and Hodgkin lymphomas (10%) this was similar to

studies done by Pagaro PM *et al.* ^[13] and Komal *et al.* ^[14] whereas in studies done by Arun Roy *et al.* ^[12] Non Hodgkin lymphomas were common followed by Hodgkin lymphomas and metastatic diseases. Among the metastatic lesions most common type was Squamous cell carcinoma (5 cases). This was similar to a study done by Pagaro PM *et al.* ^[13] and discordant with studies done by Arun Roy *et al.* ^[12] where ductal carcinoma of breast and adenocarcinoma deposits were common respectively.

In our study non Hodgkin's lymphoma is higher (16%) than Hodgkin's (10%). Most other studies by Roy A *et al.* ^[12], Ali K Ageep *et al.* ^[11] O Ochicha *et al.* ^[10] and Mbata GC *et al.* ^[9] also gave a higher preponderance of non-Hodgkin's lymphoma. The most common histological subtype, diffuse large B-cell lymphoma (DLBCL), accounts for about 37.5% of NHL.

Conclusion

The present study highlights the importance of histopathology and immunohistochemistry in lymphnode specimens for establishing the cause for lymphadenopathy. In this study reactive lymphadenitis was common followed by granulomatous lymphadenitis in non-neoplastic lesions where as metastatic diseases were common followed by Non Hodgkin lymphomas and Hodgkin lymphomas in neoplastic lesions.

Conflict of Interest

Not available

Financial Support

Not available

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