

Correlation Study of Cytomorphology, Histopathology and Immunohistochemistry in the Diagnosis of Lymphadenopathy

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ABSTRACT

BACKGROUND

Lymphadenopathy is a commonly encountered clinical entity and its study by cytological procedure is safe and minimally invasive technique, which helps in early detection and direction of appropriate investigations. Further the use of immunohistochemistry in inconclusive cases has markedly improved the final diagnosis. The purpose of this study was to evaluate the cytological and histomorphological patterns of lymphadenopathies in various age groups.

METHODS

The study was descriptive type of observational study that included 104 cases of suspected lymphadenopathies. Fine needle aspiration cytology (FNAC) was done and cytological smears were stained with standard cytological and special stains. Excisional biopsy was carried out in suspected neoplastic lesions. Biopsy sections were stained with routine haematoxylin and eosin, special stains, and immunohistochemistry (IHC) markers according to the histopathological features.

RESULTS

A total of 104 cases were studied during one year. Fine needle aspiration cytology was carried out for lymphadenopathies. Cervical lymph nodes were the most common group of lymph nodes involved with 77 cases (74.04 %). 86 cases were non-neoplastic, and 18 cases were neoplastic. In non-neoplastic lesions, maximum 28 (26.93 %) cases were of granulomatous lymphadenitis. In neoplastic lesions, maximum of 09 cases were metastasis to lymph node. Lymph node biopsy was carried out in all the suspected neoplastic cases, out of which 04 cases were reported as suspicious of neoplasia, 02 cases each were reported as metastatic adenocarcinoma and metastatic poorly differentiated carcinoma on biopsy, 05 cases of lymphomas, 03 cases of Hodgkin's lymphoma and 02 cases of non-Hodgkin's lymphoma were reported respectively.

CONCLUSIONS

Immunohistochemistry on biopsies and histopathological specimens was effective where the diagnosis was inconclusive on cytology. FNAC is a simple and cost-effective procedure in developing countries like India. FNAC has its own limitations while evaluating cases of suspected neoplasms and in sub-typing of lymphomas. To overcome these limitations, we can use lymph node biopsy to know the complete architecture and morphology of the lymph node, and to do additional studies like immunohistochemistry and immunophenotyping. Finally, this study also showed how the diagnostic efficacy is improved when cytology is combined with histopathology in the diagnosis of lymphadenopathies.

KEYWORDS

Cytopathology, Malignant Lesions, Immunohistochemistry, Diagnostic Utility

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BACKGROUND

Lymph nodes are an important integral component of the immune system and pathology of it (lymphadenopathy) is a commonly encountered clinical problem which has a multitude of causes, the most common being peripheral lymphadenopathy. Study of lymphadenopathies by cytological procedure is safe, rapid, inexpensive and minimally invasive technique, which helps in early detection and direction of appropriate investigations. FNAC is carried out as an out-patient procedure without anaesthesia for establishing diagnosis of pathological lesions occurring in lymph nodes on the exposed and easily accessible regions of the body. With radiological intervention, FNAC of inaccessible deep seated and small lesions can also be carried out. FNAC also has its own limitations like inadequate material, difficulty in very tiny lymph nodes, interpretations of benign inclusions, micro-metastasis, and inability to assess lymph node architecture. To overcome these FNAC limitations, biopsy of lymph node plays a vital role which helps to assess architecture of lymph nodes and also to do immunohistochemical studies.

Objectives

1. To study cyto-histomorphological patterns of lymphadenopathies.
2. To assess effectiveness of immunohistochemistry markers on tissue sections (biopsy and surgical specimens) where the diagnosis is inconclusive on cytology.
3. To study the efficacy of combined cytology and histopathology in diagnosis of lymphadenopathies.

METHODS

This descriptive type of observational study was undertaken after obtaining approval from Ethical Committee. The study was done on cytology smears in a private lab for over a period of one year from March 2019 to February 2020. Data was retrieved from the requisition forms of FNAC and the slides were reviewed for all 104 cases. Clinical data like age, sex, site, number of lymph nodes enlarged, single or matted nodes were noted. This study includes patients of all age groups and both genders. Fine needle aspiration was done after obtaining consent from the patients and guardians in case of paediatric patients. Patients of all age groups and both the genders who underwent FNAC of enlarged lymph nodes were included in the study. FNAC cases with inadequate material were excluded from the study.

Data Source

FNAC was performed on 104 cases of clinically suspected lymphadenopathies over a period of one year. Fine needle aspiration cytology was conducted by using 10 ml disposable syringes connected with 22 to 24 bore hypodermic needles and aspirating cytological material from lesions of lymphadenopathy. FNAC of deep-seated lesions was carried

out under ultrasound guided using lumbar puncture needle. The cytological smears prepared from the aspirate were stained with standard cytological stains. Special stains such as modified Z-N stain, PAS and Grocott's Methenamine silver were used wherever necessary. Biopsy was carried out wherever the diagnosis was inconclusive such as in cases of metastasis and in lymphomas for confirmation. Sections from formalin fixed, paraffin embedded blocks were stained with H and E stains and were studied in all cases. Special stains including Z-N, periodic acid Schiff and Gomori's methenamine silver stains were used wherever necessary. Immunohistochemical study was performed using relevant antibodies according to the histopathological features.

Statistical Analysis

Basic data was presented in percentage and diagrams wherever necessary. Chi-square test was applied to see association between different variable and P-value was calculated using SPSS 22 version. P-value < 0.001 was taken to represent significant difference.

RESULTS

A total of 104 cases of lymphadenopathy were studied during a period of one year. FNAC was done using direct methods for all visible and palpable lymph nodes and USG guidance was taken for small and non-palpable lymph nodes. Lymph node biopsy was done in cases where diagnosis was inconclusive / suspicious for malignancy and also for confirmation and subtyping of lymphomas. Out of 104 cases, 62 cases (59.62 %) were male and 42 (40.38 %) were female. Maximum 30 cases were seen between 11 - 20 years' age group followed by 27 cases between age group of 1 - 10 years age group respectively. 17 cases between 21 - 30 years, 13 cases between 41 - 50 years, 11 cases between 31 - 40 years and 6 cases above 50 years respectively.

Site	Total	Percentage %
Submandibular	14	13.47
Supraclavicular	06	5.77
Post auricular	13	12.5
Submental	03	02.88
Jugular	41	39.43
Axillary	11	10.57
Epigastric	04	03.85
Pre-aortic	03	2.89
Inguinal	09	08.64
Total	104	100

Table 1. Site Wise Distribution of Total Cases

Cervical lymph nodes were the most common group of lymph nodes involved with 77 cases (74.04 %) followed by axillary region with 11 cases (10.57 %) respectively (Table 1).

Out of 104 cases 86 cases were non-neoplastic and 18 were neoplastic respectively. In non-neoplastic lesions, maximum 28 (26.93 %) cases were of granulomatous lymphadenitis, followed by reactive lymphadenitis in 23 cases, necrotizing lymphadenitis in 19 cases, suppurative lymphadenitis in 14 cases and 2 cases of sinus histiocytosis

respectively. In neoplastic lesions, maximum 09 cases were of metastasis to lymph node followed by 04 cases of suspicious of malignancy, 03 cases of Hodgkin's lymphoma and two cases of Non-Hodgkin's lymphoma respectively. (Table 2).

	Cytomorphological (FNAC) Diagnosis	Total	ZN Stain for AFB
Non neoplastic	Granulomatous lymphadenitis	28 (26.93 %)	23
	Suppurative lymphadenitis	14 (13.46 %)	--
	Necrotising lymphadenitis	19 (18.27 %)	09
	Reactive lymphadenitis	23 (22.13 %)	--
	Sinus histiocytosis	02 (1.92 %)	--
Neoplastic	Metastatic deposits	09 (8.65 %)	--
	Hodgkin's lymphoma	03 (2.88 %)	--
	Non-Hodgkin's lymphoma	02 (1.92 %)	--
	Suspicious of malignancy	04 (3.84 %)	--
	Total	104 (100 %)	32

Table 2. Comparison of Cytomorphological Diagnosis

Z-N stain was done in 61 cases for AFB bacilli, out of which 32 cases showed positivity. Grading (1 to 4 +) and morphology of AFB bacilli into short beaded and long beaded was sub-classified further. (Table 3). In grading of AFB bacilli, 11 cases showed 4 + positivity, 10 cases showed 3 +, 7 cases showed 2 + and 4 cases showed 1 + respectively. 19 cases showed long beaded AFB bacilli and 13 cases showed short beaded AFB bacilli.

AFB		Number	
Presence	Morphology of AFB		
Positive	Short, beaded, curved and slender	1 +	2 + 3 + 4 +
	Long, beaded, curved and slender	1 +	2 + 3 + 4 +
32	13	19	04 07 10 11

Table 3. Grading and Morphology of AFB

AFB: Acid fast bacilli

On FNAC	Diagnosis On Biopsy	No. of Cases
Metastasis	Metastatic adenocarcinoma	03
	Metastatic undifferentiated carcinoma	01
	Metastatic squamous cell carcinoma	02
	Metastatic poorly differentiated carcinoma	01
	Metastatic small round cell tumour	01
Suspicious of malignancy	Metastatic seminoma	01
	Metastatic adenocarcinoma	02
Hodgkin's lymphoma	Metastatic poorly differentiated carcinoma	02
	Mixed cellularity	02
Non-Hodgkin's lymphoma	Lymphocyte rich	01
	Burkitt's lymphoma	01
Diffuse large B cell lymphoma		
		01
Total		18

Table 4. Interpretation of Neoplastic Lesions on Biopsy

Lymph node biopsy was carried out in all neoplastic cases for confirmation of diagnosis, and in cases where suspicious of malignancy was reported on FNAC and in all cases of lymphomas for confirmation and sub-typing. Four cases were reported as suspicious of malignancy on FNAC, out of which 02 cases each of metastatic adenocarcinoma and metastatic poorly differentiated carcinoma were reported on biopsy (Figure 1). One case each of metastatic small round cell tumour and metastatic seminoma was also reported in the study. Out of 05 cases of lymphomas, 02 cases of mixed cellularity Hodgkin's lymphoma, 01 case each of lymphocyte rich Hodgkin's lymphoma, Burkitt's lymphoma and diffuse

large B-cell Non-Hodgkin's lymphoma were reported respectively (Table 4) (Figure 2).

Diagnosis of lymphadenopathies according to age was studied. In younger age group, non-neoplastic lesions and Hodgkin's lymphoma were common, whereas in older age group, non-Hodgkin's lymphoma was commonly noted (Table 5).

Age (years)	0 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 +	Total
Reactive	09	07	03	02	02	00	23
Granulomatous	10	07	06	02	03	00	28
Necrotising	04	07	02	02	03	01	19
Suppurative	04	06	03	01	00	00	14
Sinus histiocytosis	00	01	01	00	00	00	02
Metastasis	00	00	01	03	04	05	13
Lymphoma	00	02	01	01	01	00	05
Total	27	30	17	11	13	06	104

Table 5. Age Distribution of the Patients with Lymphadenopathy

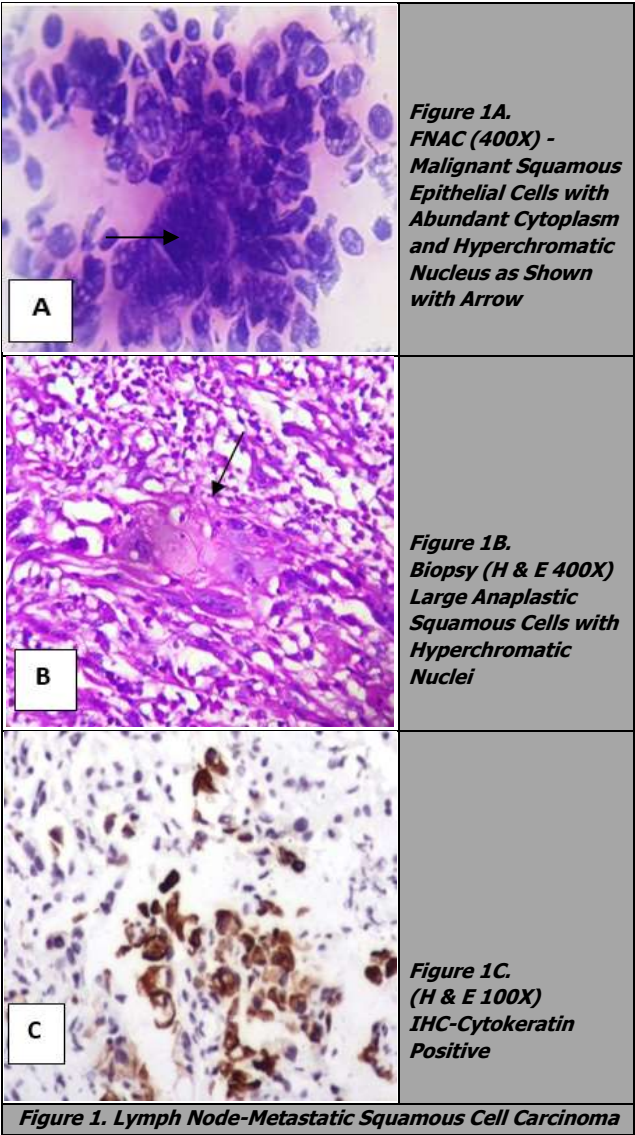
DISCUSSION

The lymphatic system plays a vital role in modulating inflammation, autoimmune disease, and anti-tumour immune response. Under pathological conditions like inflammation and in tumorigenesis. Lymph nodes undergo dramatic remodelling like changes in immune cells, cellular hyperplasia and new lymphatic vessel development. Clinical research is extremely important for more accurate diagnosis and to help evaluate the cases that require further and more specific investigation. Histological analysis is central to the assessment of these lymphadenopathies when clinical and / or radiological criteria alone are unable to establish a precise diagnosis, and especially in highly suspicious for malignancy cases.¹

The histological classification of tumours and its analysis is an important complementary technique. With the advent of immunohistochemistry, and its incorporation into routine diagnostic methods has led to improved diagnostic efficacy. Efficient antigenic recovery protocols, improved markers, lower costs and easier use of paraffin material has helped in determining more accurate histogenesis of malignant neoplastic lesions, especially in cases of poorly differentiated neoplasms, as well as for establishing and finding the primary site in cases of metastatic neoplasms. Poorly differentiated neoplasms are difficult to diagnose, and have divergent inter-examiner diagnostic rates. The use of an accurate immunohistochemical panel is essential to differentiate epithelial and mesenchymal cell lines in cases of lymphadenopathies.^{1,2}

Fine needle aspiration cytology (FNAC) is simple, safe, reliable, rapid and inexpensive method of establishing the diagnosis of lesions at various sites.² Surgical excision of peripheral palpable node is simple procedure, but it requires anaesthesia and leaves a scar. Hence, FNAC provides an alternative, immediate and almost specific diagnosis which helps in further management.³ Lymph node biopsy can be carried out when FNAC provides inadequate material or diagnosis is inconclusive. With recent advances in monoclonal antibodies, FNAC material can be used in cyto-spin preparation, cell blocks for immunophenotyping, in flow cytometry in / and for cytogenetics and molecular studies for

specific diagnosis in lymphomas and in lymph node metastasis.^{4,5}



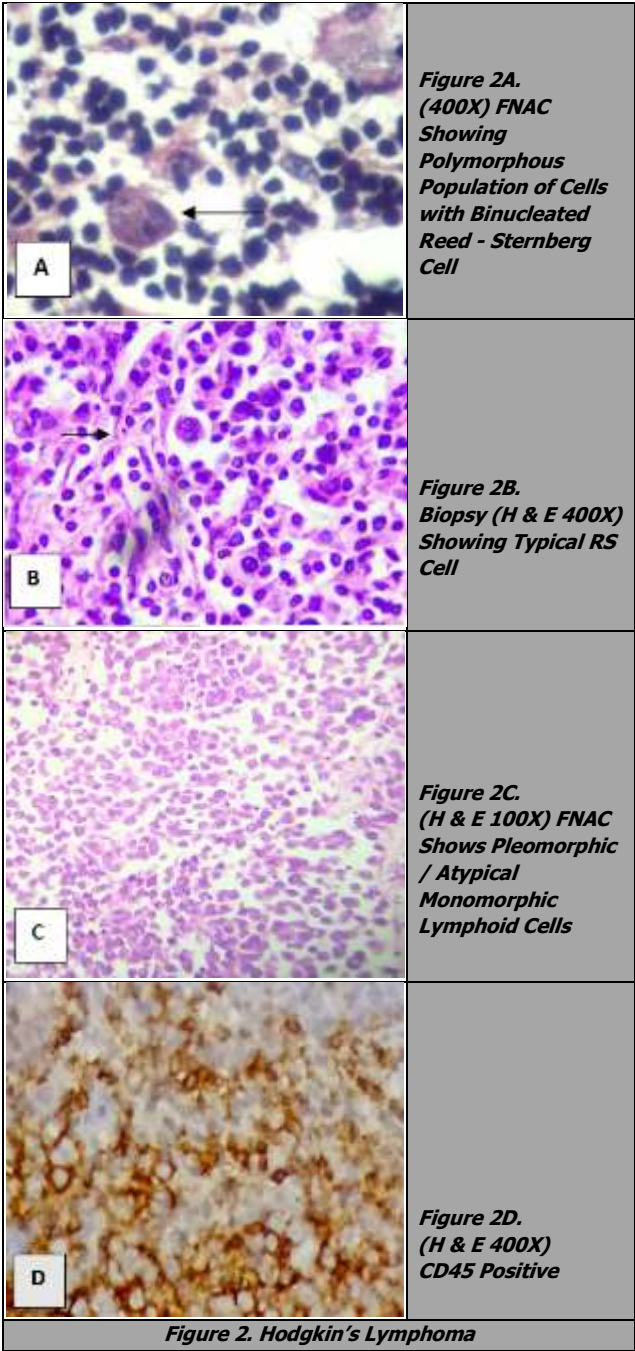
In non-neoplastic lesions, Tubercular lymphadenopathy is the most common cause of lymphadenopathy encountered in clinical practice in India. Cytological pattern of tuberculous lymphadenitis includes presence of caseous necrosis with epithelioid granulomas, with or without suppuration (necrotic material in the background with degenerating polymorphs) with AFB positivity on Z-N stained smears.⁶

The cytology of tubercular lymph node becomes more important in immunocompromised patients and also who had history of relapse or treatment failure. In this study, lymph node biopsy was done in cases of granulomatous lymphadenitis where Z-N stain was negative for confirmation.⁷

Marked proliferation of tubercle bacilli was seen in the foci of caseous necrosis and the proliferation was limited by lymphocytes, epithelioid cells and multinucleated giant cells.⁸

Hence tubercular abscess showed more AFB bacilli than the early stages of tubercular lymphadenitis. The differential diagnosis of Granulomatous lymphadenitis includes

sarcoidosis, fungal diseases, cat scratch disease, collagen vascular diseases, carcinoma, lymphoma, etc.⁹ But, in the region where tuberculosis is endemic the presence of a granulomatous feature in FNAC is highly suggestive of tuberculosis.¹⁰



ZN staining for AFB positivity in our study was 52.45 %, which was correlated well with study done by Bezabih et al. with 59.5 %. Other studies like Chand et al. reported 44.54 % and Paliwal et al. reported 71 % cases respectively. Reactive lymphadenopathy in our study was 22.11 %. Studies done by Vimal et al. and Giri et al. showed 33.64 % and 34.59 % respectively.¹¹ Acute suppurative lymphadenopathy was observed in 13.64 cases in our study. Other studies done by Patra et al. and Kocchar et al. showed 5.8 % and 4 % of cases respectively.¹¹

Enlarged lymph nodes in suspected neoplasia represent an important clinical condition that requires proper diagnosis and treatment. Squamous cell carcinomas of the head and neck have a high risk of metastasis to regional lymph nodes and the confirmation of lymph node involvement has a direct impact on rate of disease control, with marked reduction in survival curves, requiring a definitive diagnosis. Classification of lymphomas is important due to great diversity in the clinical course, changes in treatment and impact on survival rates. Thus, these days, it is practically impossible to have diagnostic conclusion without immunohistochemistry confirmation.¹²

For metastatic squamous cell carcinomas to lymph nodes panel of antibodies directed against cytokeratin 5 and 6 can be used. Major partner of CK5 is CK14, while CK6 pairs with CK16 / 17. CK5 also detected by antibodies against high molecular weight cytokeratins such as 34 β E12 and pan-CK markers such as CK AE1 / AE3. The essential features of cytokeratins is that together with p63, they can detect poorly differentiated squamous cell carcinomas. Cytokeratin like CK6 helps in assessing hyper proliferative cancer cells. Histomorphologically CK5 / 6 is expressed as diffuse cytoplasmic staining with perinuclear enhancement. They help in excluding poorly differentiated sarcomas, anaplastic lymphoma and from small round cell tumours such as neuroblastoma. IHC studies not only help in sub classifications of tumours but also helps in predicting prognosis such as diffuse positivity of CK5 / 6 is associated with poor prognosis as in triple negative breast cancers and in urothelial carcinomas.

In cases of lymphomas, FNAC plays an important role in diagnosis of Hodgkin's and non-Hodgkin's lymphomas. The cytological diagnosis of non-Hodgkin's lymphoma includes relatively monomorphic population of lymphoid cells where grading is predicted by cell size and shape, presence of nucleoli and mitotic activity.^{12,13}

Diagnosis of lymphomas by FNAC is still controversial, the reason being suboptimal cytological preparations, variable presentations and difficulty in differentiating lymphomas with reactive lymph nodes acting as major limitations.¹⁴ With the help of flow cytometry and immunohistochemistry in adjunct to FNAC, the diagnosis of NHL can be made much easier. The switch from FNAC to biopsy is more commonly seen in case of neoplastic lesions.¹⁵

One of the basic and commonly used hematopoietic marker in the diagnosis of lymphomas is CD45. CD45 also called leukocyte common antigen (LCA) plays a major role in immune system. CD45 is heavily glycosylated and expressed at high levels on nucleated hematopoietic cells. Its positivity confirms the hematopoietic nature of tumours and will assist in classification of lymphomas and leukaemias.

Metastatic malignancies in this study was compared with various other studies. Metastatic malignancies were seen in 8.65 % cases in this study. Study done by Giri et al. showed 21.8 % and Vimal et al. with 17.65 % cases respectively.¹⁶ In lymphomas, our study correlated well with 4.80 % cases with the study done by Khajuria et al. (2 %), Giri et al. (2.7 %) and Sharma et al. (2.7 %) respectively. In the present study, non-Hodgkin lymphomas and Hodgkin lymphomas

constituted 2.88 % and 1.92 % cases respectively. This was comparable to study done by Sharma et al. in which, non-Hodgkin lymphomas and Hodgkin lymphomas constituted 2.3 % and 0.4 % respectively. However, Hafez et al. reported that cases suspicious for NHL were 32.5 % and were the prominent cause of cervical lymphadenopathy.¹⁷

CONCLUSIONS

The study showed effective use of immunohistochemistry on biopsies and histopathological specimens where the diagnosis was inconclusive on cytology. FNAC is a simple and cost-effective procedure in developing countries like India. Many non-neoplastic conditions like granulomatous, necrotic and suppurative lymphadenopathies were diagnosed on cytology. But, FNAC has its own limitations while evaluating cases of suspected neoplasms and in subtyping of lymphomas. To overcome these limitations, we had done lymph node biopsy to know the complete architecture and morphology of the lymph node, and to do additional studies like immunohistochemistry and immunophenotyping. Neoplastic conditions including metastasis and lymphomas were diagnosed with accuracy on biopsies with the help of immunohistochemistry. Finally, this study also showed how the diagnostic efficacy is improved when cytology is combined with histopathology in diagnosis of lymphadenopathies.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.

Financial or other competing interests: None.

Disclosure forms provided by the authors are available with the full text of this article at jebmh.com.

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