

## The diagnostic value of FNAC in assessment of superficial palpable lymph nodes: a study of 395 cases

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**Abstract:** *Objective:* To validate the importance of FNAC in cases of lymphadenopathies by studying the different cytomorphological patterns associated with various lymphadenopathies. *Methods:* A study was carried out over a period of five years in a tertiary care teaching hospital. FNAC of 395 patients presenting with superficial palpable lymphadenopathy was reviewed, correlated with final histopathological diagnoses, wherever available, and concordance and discordance rates were evaluated. *Result:* Of the 395 patients with palpable lymphadenopathy, among 277 cases of benign lesions, reactive hyperplasia 121(30.63%) ranked on top, followed by tuberculous lymphadenitis 113(28.60%) and by pyogenic lymphadenitis 43(10.88%). And among the 113 cases of malignant lesions, 90(22.78%) cases of metastatic carcinoma were followed by malignant lymphomas 23(5.82%) cases; including NHL 15(3.80%) and HL 8(2.02%). AFB was confirmed in 78(69.02%) cases by ZN staining of FNAC smears. The nodal site sampled most frequently was cervical group of lymph nodes. Histopathology was available in 45 cases. The cytological and histological concordance was determined in 40 malignant cases. *Conclusion:* FNAC is a simple technique, easy to perform, quick and cost effective in diagnosis of lymphadenopathies which also serves as an excellent preliminary screening procedure. It obviates the need for unnecessary surgical excision of nodes.

**Keywords:** Fine-needle aspiration, lymphadenitis, lymphadenopathy, tuberculous, lymphoma, non-Hodgkins, metastasis.

### Introduction

Enlarged lymphnodes were the first organs to be biopsied by fine needle aspiration; today, they are one of the most frequently sampled tissues. Superficial lymphadenopathy is one of the commonest clinical sign encountered in the outpatient department. This may be a reaction to inflammatory process (acute/chronic) or due to primary malignant lymphoma and metastatic malignancies. Tuberculosis is among one of commonest cause of lymphadenopathies in developing countries where its prevalence is high.

It is more than 60 years since Martin and Ellis introduced aspiration biopsy in 1930 as a substitute for excisional biopsy [1]. Fine needle aspiration cytology (FNAC) was initially conceived as a means of confirming a clinical suspicion of local recurrence or metastasis of known cancer without subjecting the patient to further surgical intervention. Since then it has proven to be an acceptable, widely practiced and minimally invasive, safe, rapid and cost effective technique in establishing the diagnosis of

lymphadenopathy. Because of early availability of results, minimal trauma and complications, the aspiration cytology is now considered as a valuable diagnostic tool and is gaining popularity. Fine needle aspiration cytology has been used extensively for the diagnosis of primary malignancies as well as metastatic carcinomas involving lymph nodes and enjoys high sensitivity and specificity, of around more than 95% [2].

In patients without a previous diagnosis, not only can metastatic malignancy be confirmed by FNAC, but clues to the nature and the site of the primary are also ascertained to certain extent, and thus reduce the need for further investigations. The technique has been found most useful for the selection of a representative node for biopsy, for the diagnosis of recurrent lymphoma, for staging the extent of disease and for monitoring treatment. The clinical value of FNAC is not only limited to neoplastic condition; it is also valuable in the diagnosis of inflammatory,

infections and degenerative conditions, in which samples can be used for microbiological and biochemical analysis also. We should stress that FNAC as practiced today is still a relatively new discipline and is less demanding technologically than surgical biopsy, and thus it is eminently suitable for practice in resource scarce countries. Use of aspiration cytology is accepted as a primary method of diagnosis in reactive, infective and metastatic lymphadenopathy and in combination with immunocytochemical evaluation, flow cytometry and mRNA array studies has distinctly improved diagnostic accuracy in cases of lymphoma [3-5].

But, predominantly, cytomorphology alone decides the nature of lymphadenopathy. Knowledge about the pattern of lymphadenopathy in a population facilitates pathological reporting and helps the clinician in making focused investigation and planning the treatment course. The present retrospective study was done with the objective to validate the importance of FNAC as a first line screening procedure and diagnostic tool in cases of lymphadenopathies by studying the different cytomorphological patterns associated with various lymphadenopathies.

**Material and Methods**

Three hundred and ninety five patients presenting with superficial palpable lymphadenopathy referred to Cytopathology section, of our tertiary care teaching hospital from various clinical departments over a period of 5 years for FNAC were included in the study.

All aspirations had been performed by cytopathologists as outpatient procedure without imaging, using a 23G needle attached to 20ml syringe and Franzen handle. Half of the smears were air-dried and stained with May-Grunwald Giemsa (MGG) and the remaining half were wet fixed and stained by Hematoxylin and Eosin (H&E) and Papanicolaou (PAP) stains. Special stains like Ziehl Neelsen (ZN) for acid fast bacilli (AFB) was performed in all suspected tuberculous aspirates and Periodic acid- Schiff stain (PAS) for glycogen was done wherever required. All the stained smears were evaluated by trained cytopathologists. The cytological diagnosis was correlated with the histopathological findings, wherever available,

and concordance and discordance rates were evaluated.

**Results**

The diagnosis of 395 cases of lymphadenopathy based on cytological examination alone is shown in Table 1. Of the 395 patients with palpable lymphadenopathy, reactive hyperplasia formed the largest benign cytologic diagnostic category in 121 (30.63%) cases followed by tuberculous lymphadenitis in 113 (28.60%) cases. Metastatic carcinoma formed the major group of malignant lesions in 90 (22.78%) cases followed by malignant lymphomas in 23 cases [5.82%; Non-Hodgkin’s 15 (3.80%), Hodgkin’s 8 (2.02%)]. FNAC was inconclusive in 5 (1.27%) cases.

<b>Cytological diagnoses</b>	<b>Number of cases (percentage)</b>
<b>I. Benign lesions</b>	<b>277</b>
1. Reactive hyperplasia	121 (30.63%)
2. Tuberculous lymphadenitis	113 (28.60%)
3. Pyogenic lymphadenitis	43 (10.90%)
<b>II. Malignant lesions</b>	<b>113</b>
1. Metastatic carcinoma	90 (22.78%)
2. Malignant lymphoma	23 (5.82%)
i) Non Hodgkin’s	15 (3.80%)
ii) Hodgkin’s	08 (2.02%)
<b>III. Unsatisfactory smears</b>	<b>05</b>

The age and sex wise distribution of the patients with lymphadenopathy are summarized in Table2. It was observed that the cervical lymph nodes were involved in all types of lymphadenopathy, particularly Hodgkin’s lymphoma (HL), which showed 100% involvement of cervical group of lymph nodes. In Non Hodgkin’s lymphoma (NHL) cervical group of lymph nodes were involved in 60% of cases. The inguinal lymph nodes were least involved in all types of lymphadenopathy.

**Table-2: Age and sex wise distribution of patients with lymphadenopathy**

Age group in years	Reactive hyperplasia		Tuberculous lymphadenitis		Pyogenic lymphadenitis		Metastasis		Lymphoma			
	Male	Female	Male	Female	Male	Female	Male	Female	NHL		HL	
									Male	Female	Male	Female
0-10	45	12	11	6	3	4	0	0	0	0	0	0
11-20	20	6	3	17	3	5	0	0	1	0	2	0
21-30	4	9	12	29	6	3	1	0	0	1	0	0
31-40	5	7	3	12	4	3	1	2	1	0	3	0
41-50	3	2	6	3	3	1	16	7	2	0	0	0
51-60	2	2	3	5	3	1	24	4	3	2	1	0
> 60	3	1	0	3	2	2	31	4	5	0	2	0

**Benign lesions:** Two hundred and seventy seven nodes were diagnosed as benign lesions by FNAC. They were further grouped into three sub-categories.

**Reactive hyperplasia:** The cytosmears of these cases showed a mixed population of lymphoid cells. The cytological pattern of distribution of the cells depends on whether the follicular or intrafollicular tissue was aspirated. Thus, smears from a node containing cells of the active germinal centre had many centrocytes and centroblasts and relatively sparse mature lymphocytes, plasma cells and immunoblasts. Besides these cells, smears of reactive hyperplasia showed numerous macrophages, some with intracytoplasmic nuclear debris (tingible body macrophage). The background of these smears also showed cytoplasmic fragments, known as lymphoglandular bodies. Of the 121 cases diagnosed by cytology, histopathological examination could be done only in 5 cases, of which 3 cases diagnosed as tuberculous lymphadenitis, while one turned out to be Hodgkin’s lymphoma and one was true positive for reactive hyperplasia.

**Tuberculous lymphadenitis:** The cytological findings of epithelioid cell granuloma, Langhans’ giant cells, caseous necrosis and lymphocytes are required for the diagnosis of tuberculosis. Necrosis was seen in all the cases with epithelioid cell granuloma as the most common finding. Caseous necrosis was eosinophilic and granular and lacked recognizable cell remnants. In all these cases, cytological smears were stained with ZN stain for Acid-fast bacilli. A definite diagnosis of tuberculosis could be confirmed in

78 (69.02%) cases by AFB positivity in FNA containing necrotic material. (Table 3) Though granulomatous response is seen in a wide variety of infectious agents and non-infectious process (both benign and malignant), as tuberculosis is so common in our country, every clinically relevant case of granulomatous lymphadenitis should be considered as tuberculous lymphadenitis, unless proved otherwise. We had correlated all our cases of granulomatous lymphadenitis with the clinical presentation, Montoux test, AFB, PCR and their response to Anti-Tubercular Agents.

**Table-3: Cytomorphological features of aspirates correlated with the presence of AFB in tubercular lymphadenopathy**

Cytomorphological features	Number of cases	Presence of AFB
Epithelioid cells with caseous necrosis	77	57
Epithelioid cells, giant cells with caseous necrosis	30	21
Necrosis with polymorphs	6	None
Total number of cases	113	78

**Pyogenic Lymphadenitis:** The cytosmears showed degenerated and viable inflammatory cells, predominantly polymorphs. Repeat aspiration was advised after a course of antibiotic therapy.

**Malignant Lesions:** Malignancy was diagnosed in 28.60 % cases. Of those,

metastatic cases (79.64%) outnumbered the lymphomas (20.35%).

**Malignant lymphomas:** The presence of Reed Sternberg cells was essential to diagnose Hodgkin’s Lymphoma. In all our cases, Reed Sternberg cells were seen in the cytosmeears. Numerous atypical large mononuclear cells with prominent nucleoli were also seen. Besides these cells, variable numbers of plasma cells, lymphocytes, eosinophils, and reactive cells were seen in the background. Monotonous population i.e. single cell type predominating the smear was the most important basis for diagnosis of non-Hodgkin’s Lymphoma in cytological smears. In the present study, out of 23 malignant lymphoma

cases diagnosed by cytology only 11 cases could be available for further histopathological correlation. All the 6 cases of Hodgkin’s lymphoma and 5 cases of non-Hodgkin’s lymphoma were confirmed by histology.

**Metastatic tumors:** The most common tumor metastasizing to the nodes was the squamous cell carcinoma arising commonly in the tongue, alveolus, buccal mucosa and palate, followed by adenocarcinoma (commonest primary sites were lung, stomach, colon, ovary) and undifferentiated carcinoma. Histological correlation was possible only in 29 positive aspirates and all were concordant (Table 4).

**Table-4: Correlation between cytological and final histopathological diagnosis**

Cytological diagnosis	Number of cases	Histopathological diagnosis			
		Reactive hyperplasia	Tuberculosis	Metastasis	Lymphoma
Reactive hyperplasia	5	1	3	None	1
Tuberculosis	None	None	None	None	None
Metastasis	29	None	None	29	None
Lymphoma	11	None	None	None	11
Total cases	45	1	3	29	12

### Discussion

Cytology of lymph nodes has become a window for diagnosis of many diseases. Optimal material and experience, when combined, make cytological diagnosis of equal significance as histopathology. In many clinical settings it is very difficult to decide which patient is more likely to have a reactive or neoplastic lymphadenopathy. Here, knowledge about the pattern of lymphadenopathy is helpful to the clinician for solving the dilemma. The common causes of peripheral lymphadenopathy are either a reaction to inflammatory process or metastatic carcinoma/malignant lymphoma. Using FNA cytology it is often possible to decide the etiology of lymphadenopathy and may also point towards a possible occult primary site in case of secondaries with unknown primaries.

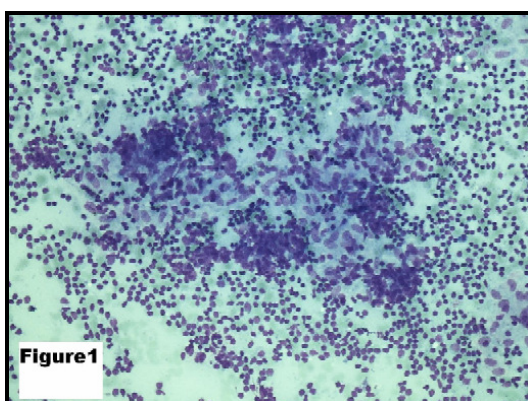
We are presenting our experience with 395 cases of lymphadenopathy gathered over a period of five years. The pattern of benign lesion seen in

our study consists of reactive hyperplasia, tuberculosis, pyogenic lymphadenitis and, malignant lesion includes metastatic carcinoma and malignant lymphoma. In the present study of the 395 patients, 277 (70.12%) cases were benign and 113 (28.60%) cases were of malignant lesion which was nearly similar to previous studies [6-8]. However, Steel et al reported 59% cases of malignant lesions and 34% cases of benign lesions [9]. This may be attributed to the fact that western countries, where this study was carried out, show predominance of malignant over benign conditions. In our study, bulk of diseases were of reactive nature due to infections and tubercular lesions, which are uncommon in western countries [10]. Among the benign lesions, reactive hyperplasia 121 (30.63%) ranked on the top, followed by tuberculous lymphadenitis 113 (28.60%) followed by pyogenic lymphadenitis 43 (10.88%) and among the malignant lesions

metastatic carcinomas 90 (22.78%) was followed by malignant lymphomas 23 (5.82%) These findings were comparable with those described in a study of 2418 cases [11].

Lymph nodes can be detected in patients ranging from early to advanced age. In our study, the youngest patient was 1.5 years old and oldest was 90 years of age. These figures come in close comparison to a previous study where the youngest patient was 1 year old and oldest was of 90 years [9]. We observed the peak incidence of benign lesions in first two decades (34.17%), while the malignant lesions (25.56%) in the sixth decade, which have been reported in a previous study [6]. It was also observed that males showed preponderance of reactive hyperplasia, lymphoma and metastatic carcinoma while in females tuberculous lymphadenitis was the most common diagnosis which is in agreement with the other researchers in their studies on lymph nodes [12-13]. We observed that the most common site of involvement was cervical group of lymph nodes in 91.89% of cases, which was followed by axillary group and inguinal group of lymph nodes in 3.29% and 2.78% cases respectively, which is quiet similar to observation made by other study groups [13-14]. In India, tuberculous lymphadenitis is one of the most common types of lymphadenopathy encountered in clinical practice [11], whereas it is in sharp contrast to very low frequency of 1.6% in western studies [15]. In our group of patients, it formed the second largest group, most frequent in second and third decade of life (53.98%) with female preponderance which substantiates the observation made in other studies [16-17].

**Figure-1: Photomicrograph showing tubercular granuloma – loose aggregates of epithelioid histiocytes (MGG Stain; x 400)**

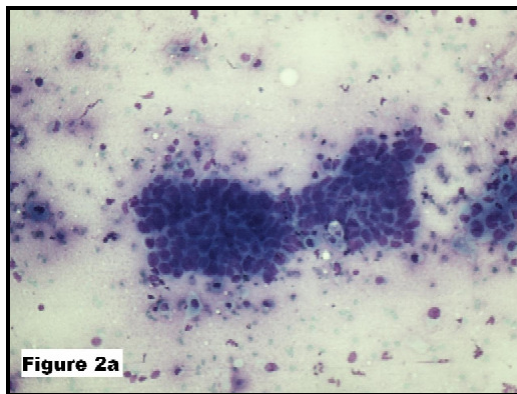


Early diagnosis is particularly important in tubercular lymphadenopathy due to its curability. The cytological diagnosis of tubercular lesions depends upon demonstration of either epithelioid cell granuloma with caseous necrosis with or without Langhan's giant cell, with necrosis in a milieu of lymphoid cells or, only necrotic material consisting of diffuse granular debris. Epithelioid cell granulomas (Fig.1) were seen in 107 cases and Langhan's giant cells were identified in 30 cases. Necrosis was seen in all the cases. The diagnosis of tuberculosis can be made with confidence by isolating the bacilli by culture or, visualizing the organism. In our study we could confirm presence of AFB in 78 (69.02%) of these patients, this percentage was higher in contrast to other studies [16,18-19]. We observed that AFB was usually found extracellularly in the areas of necrosis, within or at the periphery of epithelioid cell granulomas. These findings correlated with those given by Rajsekaran et al in their study on tubercular lymph nodes [19].

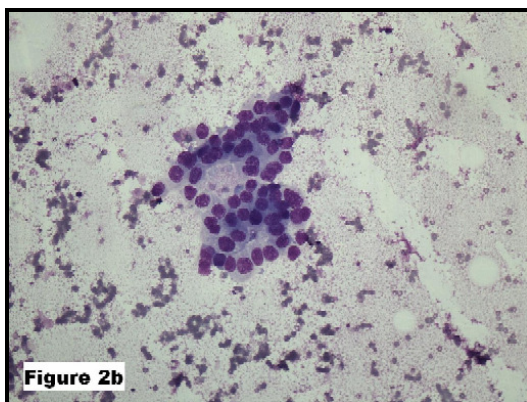
Cytomorphologic features of the aspirate are important for the detection of AFB in the tubercular lymph nodes. In FNAC smears, the presence of necrosis should alert one to the diagnosis of tuberculosis. Therefore, with our experience we feel that all smears/aspirates obtained by FNAC of suspected tuberculosis shall be subjected to ZN staining for AFB. We observed that AFB are mostly visible in purulent aspirate whether acellular or, accompanied by well defined granuloma and in absence of ZN staining can be misinterpreted as suppurative lymphadenitis. But retrospectively, it seems to us that it must have been prudent to subject AFB negative but still suspicious cases, to repeat FNAC, for culture and sensitivity. Lymph nodes are the most common site of metastatic malignancy and sometimes constitute the first clinical manifestation of the disease. The task of pathologist is to identify the presence of a malignant process in the node, and to establish whether it is metastatic or, not. Fine needle aspiration of lymph nodes is particularly useful for the documentation of metastatic carcinoma and can obviate further surgery performed merely to confirm the presence of metastasis. In our group of patients, metastatic

malignancy was diagnosed in 90 patients and the most common tumors in order included, squamous cell carcinoma (Fig.2a), adenocarcinoma (Fig.2b) and undifferentiated carcinoma. The highest incidence (95.55%) was seen in over 40 years of age with the male preponderance. Most commonly involved sites were cervical group of lymph nodes (97.77%). Similar results have been reported in previous studies [20-22]. FNAC proved to be a useful tool in diagnosing metastasis with good certainty. It helps in planning surgery for malignant cases, where definitive operative intervention can be performed in one session. A thorough study of the morphological details of the individual metastatic tumor cells can help in suggesting the most likely primary sites of tumor.

**Figure-2(a): Photomicrograph showing metastatic squamous cell carcinoma (MGG Stain; x 400)**



**Figure-2(b): Photomicrograph showing metastatic adenocarcinoma (MGG Stain; x 400)**



Although FNAC does not replace histological examination in the diagnosis of lymphoma, it is still of value in diagnosis, grading/ classification, in the management of lymphoma, and therefore FNAC has been used extensively in cases of NHL

and HL. There were 23 cases of malignant lymphoma, 15 NHL and 8 HL. Cases of lymphoma were distributed in all age groups but 80% of NHL was over 40 years of age with a male preponderance as reflected in a previous study [23]. Though there prevalence is low, they pose a great diagnostic challenge. One of the main limitations to FNAC as an independent diagnostic tool for lymphoma has been the inability to subclassify cases for directing appropriate therapy, given the reliance of previous classifications on architectural features. With advances in the understanding of the biology, immunology and molecular genetics of lymphoma in the past two decades, lymphoid neoplasms are now defined by a combination of morphologic, immunophenotypic, genotypic features, and characteristic clinical presentations. Architectural patterns, which historically have been the gold standard for lymphoma subclassification, are no longer of paramount importance in many cases. As a result, it should now be possible to diagnose and classify lymphoma subtypes that have a characteristic cytomorphology and immunophenotype without examination of tissue sections.

Immunophenotyping by flow cytometry (FCM) is a rapid and sensitive method to evaluate lymphoid markers and is critical for lymphoma diagnosis and subclassification, particularly in the areas of small cell lymphomas, which have overlapping morphology both with reactive process and with each other, making diagnosis and subclassification extremely difficult on cytomorphology alone [4]. FCM appears to be extremely suitable for cytology specimens because it requires only a small sample, is suitable for cells already in suspension, and has a rapid turnaround time. Dong et al in a recent series, reviewed 139 consecutive FNAC of 84 primary and 55 recurrent lymphomas, with and without ancillary FCM analysis to diagnose and subclassify these tumors according to the Revised European-American Lymphoma/WHO classifications. They found that cytomorphology coupled with FCM analysis provided a definitive diagnosis of lymphoma in 77% cases with similar accuracy rates for primary and recurrent



lymphomas; without FCM, the accuracy rates was 67%, supporting the importance of FCM as an important adjunct to the cytological diagnosis and subclassification of lymphoma [5]. For determination of sensitivity, specificity and diagnostic accuracy of FNAC, a correlation of cytodiagnosis with subsequent histological reports is required. In the present study, of the 395 cases diagnosed by cytology, histopathological examination could be done in 45 cases. A majority of patients presented with reactive hyperplasia and tuberculosis, not mandating biopsy, still suspicious cases were subjected to histopathological examination. However, such a sample size is insufficient to conclusively comment upon the sensitivity and specificity. Although previous studies with greater number of cytohistological correlations have reported high sensitivity, specificity and diagnostic accuracy [8, 21, 23]. All the 40 malignant lesions (29 metastatic carcinoma and 11 lymphoma) diagnosed on cytology were in concordance with histopathological diagnoses. This reinforces that sensitivity and specificity of FNAC for malignancy is high enough to be used as a diagnostic tool. In the present study, FNAC was inconclusive in 5 cases (1.2%) due to unsatisfactory smears. These included cases in which aspirated material was either inadequate or smears were unsatisfactory due to poor preparation and/or staining.

Using cytomorphology alone it is often possible to decide whether the lymphadenopathy has resulted from reactive hyperplasia, metastatic malignancy or lymphomas. Thus patients with reactive lymphnode enlargement or metastasis from a known primary can be spared lymphnode biopsy. Today, this seems to be the prevailing application of FNAC in patients with

lymphadenopathy, but still we feel that it could be improved upon by using special techniques like immunocytochemistry which was lacking in our setup. There is however been a trend towards accepting cytomorphology alone as sufficient for diagnosis in patients with abdominal or mediastinal lymphomas. To conclude, with the increasing costs of medical facilities, any technique which speeds up the process of diagnosis limits the physical and the psychological trauma to the patient and saves the expenditure of hospitalization, is of tremendous value. FNAC is a simple technique- easy to perform, quick and less expensive in diagnoses of lymphadenopathies, which not only offers tissue diagnosis but serves as a preliminary screening procedure for a number of clinical considerations e.g. lymphoma, leukemia, metastasis, tuberculosis etc. The high specificity of technique helps to single out those that need further investigation or biopsy and obviates the need for the unnecessary surgical excision of nodes. Moreover, the procedure is very cost effective, free of complications, well tolerated by the patient, done on an outpatient basis and repeatable. Aspiration cytology is now accepted and successfully employed as a diagnostic technique by a number of institutions in a resource scarce country like India. FNAC has proved to be advantageous, economical and convenient alternative to surgical biopsy of lymph nodes; whose diagnostic accuracy has been further improved manifold when used in collaboration with other special techniques like immunocytochemistry, ultrastructural studies, cytogenetic parameters and molecular hybridization.

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