

A Correlative Cytological and Histopathological Study on Superficial Lymphadenopathy

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Abstract: The lymph node is one of the major anatomic components of the immune system. Lymph nodes are the most widely distributed and easily accessible component of lymphoid tissue and hence they are frequently examined for diagnosis of lymphoreticular disorders. **Objectives:** To assess the usefulness of the cytological study in the diagnosis of lymph node lesions, To evaluate the accuracy of FNAC studies in correlation with histopathological study. **Study design:** cross sectional study. **Specimens obtained from patients having lymph node enlargement (cervical, inguinal, axillary) of Govt Rajaji Hospital Madurai Medical College, Madurai were included in the study. FNAC was performed 388 patients, Out of which 87 patients had histo pathological correlation, The overall sensitivity for FNAC of superficial lymphadenopathy was 91.6%, specificity was 98.3%, and accuracy was 97%. The result are quite encouraging and FNAC is recommended as the initial diagnostic test in the evaluation of superficial lymphadenopathy. Although F evaluation of superficial lymphadenopathy. Although FNAC has proven to be a simple, safe, reliable and cost effective diagnostic tool for lymphadenopathies, the limitation of the procedure should be kept in mind and excision biopsy should be used whenever required. Immunohistochemical staining is useful for confirmation of diagnosis in nodal lesions especially lymphoma and for further classification of lymphoma**

Keywords: FNAC, Histo pathology, correlation, superficial lymphadenopathy

1. Introduction

The lymph node is one of the major anatomic components of the immune system. Lymph nodes are the most widely distributed and easily accessible component of lymphoid tissue and hence they are frequently examined for diagnosis of lymphoreticular disorders.

Diagnosis of lymphadenopathy depends mainly on excision of a gland and histopathological examination. For this, general anaesthesia and hospitalisation are required. Fine needle aspiration cytology, on the other hand, is free from these disadvantages and can safely be used as an alternative or complementary investigative technique.

Grieg and Gray¹ used aspiration of lymph nodes as early as in 1904 for the diagnosis of sleeping sickness by recognizing mobile trypanosomes.

Guthrie in 1921² compiled one of the earliest series on aspiratin biopsy from cervical lymphadenopathy, describing cell specimens from lymphadenitis, metastatic carcinoma, and Hodgkin's disease. Forkner³ in 1927 studied node punctures from 30 patients, and Martin and Ellis⁴ in 1930 aspirated from lymph nodes in 23 cases.

FNA biopsy rarely interferes significantly with histological interpretation⁵ FNA biopsy can be useful to obtain material for culture, because a specific diagnosis can lead to specific therapy⁶. A normal lymph node is rarely palpable. Cytological characteristics of cells from a normal lymph node are essentially based on the morphology of individual cells as observed in the aspirate from a reactive lymph node. The lymphocytes constitute 87% to 99%, Plasma cells 0% to 5% and remainder cells (histiocytes, mast cells, eosinophils and neutrophils) 1% to 3%.

The aim of the study was to evaluate the accuracy of FNAC studies in correlation with histopathological study, To compare the sensitivity, specificity and accuracy with

previous studies conducted at other centres, To determine and evaluate the causes for false positivity and negativity and to arrive at possible suggestions to minimize the percentage, in this regard.

2. Material and Methods

The present study was carried out in the Department of Pathology, Madurai Medical College, Madurai, India for a period of 2 years.

The cytological materials were obtained in the form of smears, which were fixed in 95% alcohol for PAP and H&E staining. The aspiration syringes used were 10-20ml and needle size between 22-23 gauges.

The specimens were received from Department of Surgery, Government Rajaji Hospital, Madurai. Details of the patients such as clinical and personal history were recorded including details of operative findings, macroscopic and microscopic features.

The histopathology specimens were fixed in 10% buffered formal saline. After paraffin embedding 5 micron thick sections were made, stained with H&E and PAP. Special stains such as Reticulin were used as and when required. Immuno histochemical studies were done in relevant cases.

The results of the lymph node were divided in the following diagnostic categories: Reactive lymphadenitis, Tuberculous Lymphadenitis, Lymphomas and metastatic deposits.

A suspicious report was issued when there were only few abnormal cell or cells lacking the majority of criteria for malignancy. Specimens were considered unsatisfactory when there were sparse lymphoid elements, poor cell preservation or much blood. The results of both procedures were compared. The formulae for assessing sensitivity and specificity are as follows:

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Screening test results	Diagnosis		Total
	Diseased	Not Diseased	
Positive	a(True Positive)	b(False Positive)	a+b
Negative	c(False negative)	d(True negative)	c+d
Total	a+c	b+d	a+b+c+d

The following measures are used to evaluate a screening:
 Sensitivity= $a / (a+c) \times 100$
 Specificity= $d / (b+d) \times 100$
 Accuracy= $a+d / (a+b+c+d) \times 100$.

3. Observation and Results

During the study period, 388 smears were received for cytological examination. Out of these 87 cases had post surgical follow-ups.

Out of the 87 smears studied, 21 cases (24.1%) were reactive lymphadenitis, 26 cases (29.9%) were Tuberculous lymphadenitis, 6 cases (6.9%) were Non Hodgkin's Lymphoma, 3 cases (3.4%) were Hodgkin's Lymphoma, 30 cases (34.5%) were Metastatic deposits and one case was (1.2%) found to be unsatisfactory. (Table 1)

The commonest non-neoplastic lesion encountered was tuberculous lymphadenitis. The most common malignant lesion diagnosed was metastatic carcinomatous deposits.

Table 1: Details of lesions found in FNAC of Lymph node

FNAC Diagnosis	Number of Cases	Percentage (%)
Reactive Lymphadenitis	21	24.1 %
Tuberculous Lymphadenitis	26	29.9%
Non Hodgkin's Lymphoma	6	6.9%
Hodgkin's Lymphoma	3	3.4%
Metastatic Deposits	30	34.5%
Unsatisfactory	1	1.2%

Histopathological Diagnosis:

The histopathological diagnosis offered for the 87 cases is shown in table 2. Out of the 87 cases 20 cases (23%) were reactive lymphadenitis, 29 cases (33.3%) were Tuberculous lymphadenitis, 4 cases (4.6%) were Non Hodgkin's Lymphoma, 4 cases (4.6%) were Hodgkin's Lymphoma, one case (1.2%) was Leukemia involving the lymph node and 29 cases (33.33%) were metastatic deposits. The most common non-neoplastic lesion encountered was tuberculous lymphadenitis. Among the malignant lesion, most common was metastatic carcinomatous deposits.

Table 2: Details of lesions on Histopathological Examination of Lymph node

HP Diagnosis	Number of cases	Percentage (%)
Reactive Lymphadenitis	20	23%
Tuberculous Lymphadenitis	29	33.3%
Non Hodgkin's Lymphoma	4	4.6%
Hodgkin's Lymphoma	4	4.6%
Leukemia involving the Lymph node	1	1.2%
Metastatic Deposits	29	33.33%

Correlation between cytological and histopathological diagnosis

Out of the 21 smears of reactive lymphadenitis, 18 were diagnosed as reactive lymphadenitis by histopathology, 2

were diagnosed as tuberculous lymphadenitis, and one case was diagnosed as Hodgkin's Lymphoma. All the 26 smears of tuberculous lymphadenitis were confirmed by histopathology.

Out of 6 smears of Non Hodgkin's Lymphoma, only 4 cases were diagnosed by histopathology, one case turned out to be a reactive lymphadenitis and one case was turned out to be a Leukemia involving the lymph node. All the 3 smears of Hodgkin's Lymphoma were confirmed by Hodgkin's Lymphoma by histopathology. Out of 30 smears of metastatic deposits, 29 were diagnosed as same by histopathology and one was diagnosed as reactive lymphadenitis.

There was an excellent correlation in cases of tuberculous lymphadenitis (100%) and Hodgkins Lymphoma (100%).

Table 3 illustrates the correlation obtained between cytological and histopathological diagnosis.

Table 3: Correlation between cytological and histopathological diagnosis

FNAC Diagnosis	No of cases	Reactive	TB	NHL	HL	Leukemia	Metastatic
Reactive	21	18	2	---	1	---	---
TB	26	---	26	---	---	---	---
NHL	6	1	---	4	---	1	---
HL	3	---	---	---	3	---	---
Leukemia	0	---	---	---	---	---	---
Metastatic	30	1	---	---	---	---	29
Unsatisfactory	1	---	1	---	---	---	---

4. Sensitivity and Specificity

Reactive Lymphadenitis

Out of 21 cases, which were diagnosed cytologically as reactive lymphadenitis, 18 cases turned out to be reactive process (True Positive-18) and 2 cases were tuberculous lymphadenitis and one case turned out to be a Hodgkin's Lymphoma. (False Positive-3) The sensitivity of FNAC in diagnosing reactive lymphadenitis was found to be 90%. One case, which was diagnosed as metastatic deposits and other case that was diagnosed cytologically as lymphoma turned out to be a reactive lymphadenitis histopathologically. Hence, specificity for this lesion is 95.6% and accuracy was 93.1%.

Tuberculous Lymphadenitis

26 cases, which were diagnosed as tuberculous lymphadenitis cytologically were confirmed by histopathology also. (True Positive-26). Two cases, which were diagnosed as reactive lymphadenitis cytologically, turned out to be tuberculous in histopathology (False Negative-2). The sensitivity, specificity and accuracy rates were 92.8%, 100%, and 96.5% respectively.

Non-Hodgkin's Lymphoma

Out of 6 cases diagnosed cytologically, only 4 were confirmed by histopathology (True Positive-4). One case turned out to be a Leukemia involving the lymph node and other turned out to be a reactive lymphadenitis. The sensitivity of Non-Hodgkin's Lymphoma was 100%, the

specificity was 97.5%, and accuracy was 97.7%. 3 out of 4 cases had bone marrow involvement. Immunohistochemical staining showed positive for CD20 in three cases of Non Hodgkin's Lymphoma.

Hodgkin's Lymphoma

Only 3 cases were diagnosed cytologically as Hodgkin's Lymphoma, and all the three cases were confirmed histopathologically also (True Positive-3). However, one case which was diagnosed histopathologically as Hodgkin's was diagnosed cytologically as reactive lymphadenitis (False negative-1). Hence, the sensitivity of FNAC in Hodgkin's Lymphoma was 75%, whereas specificity was 100% and accuracy was 98.6%. Immunohistochemical markers CD15 and CD30 were positive in a case of Hodgkin's Lymphoma mixed cellularity type

Metastatic Deposits

Out of 30 cases diagnosed as metastatic deposits cytologically, 29 cases were confirmed histopathologically (True Positive-29), One case turned out to be reactive Lymphadenitis (False Positive-1). The sensitivity of metastatic deposits by FNAC is 100%, specificity was 98.2%, and accuracy was 98.8%.

Thus, overall sensitivity, specificity and accuracy of F.N.A.C in superficial lymphadenopathy were 91.6 %, 98.3%, 97 % respectively. The details of sensitivity, specificity and accuracy of each lesion were illustrated in Table 4.

Table 4: Details of Sensitivity, Specificity and Accuracy of lymph node lesions

Diagnosis	Sensitivity	Specificity	Accuracy
Reactive Lymphadenitis	90%	95.6%	93.1%
Tuberculous Lymphadenitis	92.8%	100%	96.5%
Non Hodgkins Lymphoma	100%	97.5%	97.7%
Hodgkins Lymphoma	75%	100%	98.6%
Metastatic Deposits	100%	98.2%	98.8%

5. Discussion

The recent trend in medical practice is toward adopting a diagnostic modality, which is both cost effective and minimally invasive. In this regard, FNAC is often used as a first line of investigation for screening cases with lymphadenopathy, since this method is easy to perform, rapid, and inexpensive. FNAC can help not only to differentiate among lymphoma, and metastasis, but also to identify nonspecific reactive lymphadenitis and specific infections such as tuberculous lymphadenitis.

Reactive Lymphadenitis

The cytological features of reactive lymphadenitis revealed cellular specimens composed of small lymphocytes predominated with a few neutrophils, plasma cells, histiocytes elongated fibroblasts and phagocytes.

Grossly the affected lymph nodes were enlarged and firm. Histo pathologically reactive lymphadenitis showed follicular, sinus, or mixed patterns.

In follicular hyperplasia, histopathological examination showed marked enlargement and prominence of the germinal centers. The results of this work indicate that benign lymphadenopathy constitutes a significant proportion of findings in aspirates of enlarged lymph nodes (55.3%). It was also proved that cytological examination may not only help to distinguish between benign and malignant types, but may also suggest the nature of the benign process. Out of non-neoplastic lymph node lesions which formed 55.3%, the second most common diagnosis was reactive hyperplasia, which was second to the incidence of tuberculosis in the present study. This figure is correlating with the study of Bezabih M et al (2003)⁸

One case, which was diagnosed as metastatic deposits, and other case that was diagnosed as lymphoma by cytology were turned out to be a reactive lymphadenitis (False Negative -2). These errors were made because of cell with large nucleoli. Some were histiocytes, later recognized because of its size and chromatin pattern. Others, immature lymphocytes from the germinal centers, still have potential for causing misdiagnosis. Errors can be diminished by noting surrounding benign polymorphic cells and occasional phagocytes.

The sensitivity of diagnosing reactive lymphadenitis is 90%

Tuberculous lymphadenitis

The cytological diagnosis of tuberculous lymphadenitis was made definitely, when granulomas composed of epithelioid cells and Langhans' cells are seen. but even in the absence of granulomas, necrosis along with the presence of lymphocytes alone gives an indirect evidence of tuberculous lymphadenitis.

Grossly the affected lymph nodes are matted and showed caseous necrosis.

Histopathological examination of tuberculous lymphadenitis showed caseous necrosis, epithelioid cell granulomas and Langhans' giant cells.

In the present study, the sensitivity for tuberculous lymphadenitis is 92.8%. Two cases of tuberculous lymphadenitis diagnosed as reactive lymphadenitis on cytological examination were subsequently found to be tuberculous lymphadenitis in histopathology. Probably, the representative samples were not obtained in these cases.

The diagnostic accuracy in the present study is 96.5%. This is higher than accuracy rate of other research workers. The success result of FNAC in tuberculous lymphadenitis by other research workers were 80% (Bloch, 1967)⁹; 87.18% (Patra et al, 1983)¹⁰; and 83.33% (Dandapat et al, 1986)¹¹.

Non Hodgkin's Lymphoma

Cytological criteria for diagnosing Non Hodgkin's Lymphoma were monomorphism and macronucleoli. Although sub classification was attempted, the aim was to distinguish lymphoma from hyperplasia.

Grossly cut surface of enlarged lymph node showed gray white and fish flesh appearance.

Histopathological examination of Small Lymphocytic Lymphoma shows loss of architecture and replaced by diffuse proliferation of well differentiated, mature, small and uniform lymphocytes without cytologic atypia or significant mitoses.

Histopathologically Diffuse Large cell Lymphoma have diffuse pattern of involvement by large cells showing round to oval nucleus and two to three nucleoli, with increased mitotic activity.

Reticulin stain in a case of Non Hodgkin's Lymphoma showed a fine, branching reticulin network with pericellular fibrils characterize the lymphoma.

Immuno histochemically three of Non Hodgkin's Lymphoma cases showed CD 20 positivity.

The diagnostic accuracy of Non Hodgkin's lymphoma is 97.7% Aspirate of one patient was false-positively interpreted as lymphoma, who had a corresponding biopsy showing reactive hyperplasia. This error was made because of the presence of a few cells with large nucleoli. Some of these cells were histiocytes and others were immature lymphocytes from the germinal centers. Lee et al also found difficult in differentiating between reactive hyperplasia and lymphoma by cytology alone.

Hodgkin's Lymphoma

In Hodgkin's lymphoma, the main cytological feature was polymorphism constituted by immature lymphocytes, eosinophils and Reed-Sternberg cells.

Grossly the cut surface of Hodgkin's lymphoma showed nodular scarring in nodular sclerosis type whereas mixed cellularity type showed abundance of necrosis.

On microscopic examination of nodular sclerosis type of Hodgkin's lymphoma showed variable amount of fibrous tissue and characteristic lacunar type of RS cells.

Immuno histochemical markers CD15 and CD 30 were positive in a case of Hodgkin's Lymphoma mixed cellularity type.

The diagnostic accuracy of Hodgkin's lymphoma is 98.8%. One case was diagnosed as reactive lymphadenitis in cytology, but showed Hodgkin's lymphoma in histopathology. This may be due to absence of Reed-Sternberg cells in the cytology smears.

During the study period, one case was diagnosed histopathologically as reactive lymphadenitis. After six months, the same case was rediagnosed as suggestive of Hodgkin's Lymphoma cytologically and that was confirmed by histopathology also.

In False negative cases, it may be necessary to repeat FNAC studies, if there is strong clinical suspicious of neoplasm. Sometimes False negative results can be encountered due to small or inaccessible nodes, fibrosis, necrosis or sampling the wrong node.

Gupta et al (1977)¹² who exclusively studied aspiration smears of lymphoma cases also mentioned the possibility of overlap among reactive hyperplasia, lymphocytic lymphoma and Hodgkin's disease.

Gupta et al (1977)¹², Tripathi et al (1985)¹³, Mondal et al (1989)¹⁴ and Al-Muhim et al(2004)¹⁵ quoted the diagnostic accuracy in lymphoma as 84%, 80%,96.2% and 88% respectively. In the present study, the diagnostic accuracy was 97.7%.

Leukaemia involving Lymph node

Histopathologically Acute Lymphoblastic Leukaemia involving the lymph node was appearing to begin at the center of the node residual lymph node structure was seen at the periphery.

One case was reported as suggestive of lymphoma in F.NAC, tissue section of the same lymph node when correlated with blood smear findings, revealed evidence of leukemia involving the lymph node. In such cases, it is very difficult to differentiate between Non-Hodgkin's lymphoma and leukemic infiltration on the examination of cytological smears alone. Hence, in all cases of suspected lymphoma, details regarding haematological and smear findings and marrow features are essential for correlative study.

Metastatic Deposits

The FNAC from metastatic carcinoma was cell rich chiefly with cells foreign to lymph nodes, but occasionally, intermingled with lymphoid cells. Specific primary site identification was accurate in many cases.

Cells from squamous cell carcinoma, papillary adeno carcinoma and malignant melanoma were the easiest to identify. Cells from squamous cell carcinoma were single, pleomorphic and had abundant homogeneous keratinized cytoplasm with central nuclei. Papillary clusters, intranuclear clear areas and rare psammoma bodies were characteristic features of papillary thyroid carcinoma . Metastatic melanoma showed features of isolated oval cells, two or three distinct cell populations based on cell size, multinucleation, macro nucleoli, and intra nuclear inclusions.

Grossly metastatic deposits of malignant melanoma showed brownish melanin pigment deposits. Metastatic deposits of squamous cell carcinoma showed grayish white tumour areas.

Histopathologically, in malignant melanoma lymph node was partially replaced by melanoma cells showing marked pleomorphism and prominent melanin pigments. Reticulin stain of squamous cell carcinoma showed thick reticulin fibres surrounding the cords and islands of carcinoma cells.

During the study period, metastatic squamous cell carcinoma was found in maximum number (41.3%) followed by adenocarcinoma (27.6%). This is correlating with the study of Hema arora et al.¹⁶

In the present study the accuracy rate of diagnosing metastatic carcinoma is 98.8% It is higher than the accuracy reported by Gupta et al (90%)¹² and Tripathi et al (80.2%)¹³.

In the present study overall sensitivity, specificity and accuracy of F.N.A.C in superficial lymphadenopathy were 91.6 %, 98.3%, 97 % respectively.

Table 6 shows the comparative study of accuracy of individual lesions with various authors.

Table 7 shows the comparative study of sensitivity, specificity and accuracy of F.N.A.C in superficial lymphadenopathy with various studies.

Table 6: Comparison of Diagnostic accuracy of individual lesions

Author	Reactive	TB	NHL	HL	Metastatic	Overall accuracy
Mondal A et al (1989) ¹⁴	97	100%	92.3%	100%	98.4%	96.2%
Al Muhlim et al (2004) ¹⁵	100%	93%	86%	90%	91%	93%
Current Study	93.1%	96.1%	97.7%	98.6%	98.8%	97%

Table 7: Comparative Study of Sensitivity, Specificity & Accuracy observed in various studies on lymph node lesions

Author (Year)	Sensitivity	Specificity	Accuracy
Lioe TF et al (1999) ⁷⁰	85.4%	100%	94.4%
Van de School et al (2001) ¹²⁵	92%	90%	-
Gupta RK et al (2003) ⁴⁰	92.7%	98.5%	96.%
Stiegler R et al(2003) ¹¹⁶	97.6%	96.0%	-
	91.6%	98.3%	97%

6. Conclusion

“A correlative cytological and histopathological study on superficial lymphadenopathy” revealed the overall sensitivity was 91.6%, specificity was 98.3% and accuracy was 97%. False positive and false negative reports can be minimized by sampling appropriate nodes and by correlative haematologic study.

The results are quite encouraging and FNAC is recommended as the initial diagnostic test in the evaluation of superficial lymphadenopathy. Although FNAC has proven to be a simple, safe, reliable and cost effective diagnostic tool for lymphadenopathies, the limitation of the procedure should be kept in mind and excision biopsy should be used whenever required. Immunohistochemical staining is useful for confirmation of diagnosis in nodal lesions especially lymphoma and for further classification of lymphomas.

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