FINE NEEDLE ASPIRATION CYTOLOGY OF LYMPH NODES WITH HISTOLOGICAL CORRELATION- A STUDY OF 1033 CONSECUTIVE CASES, A SOUTH INDIAN STUDY

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ABSTRACT

BACKGROUND

Accurate diagnosis of lymphadenopathy is of utmost importance in planning proper therapeutic measures. This is possible with a multidimensional view of the problem facilitated by understanding of its clinical appearances, cytology, histology and other lab investigations before making a diagnosis.

Aims and Objectives- To evaluate the role of FNAC in diagnosis and management of lymphadenopathy- changing perspectives in view of better cytodiagnosis and fewer biopsies.

MATERIALS AND METHODS

All patients presenting to the cytology section with lymphadenopathy and reference material submitted to the Department of Pathology, KIMS, Hubli for cytopathological study during the period from December 2010 to May 2012 were included. FNAC was done using 23-gauge needle. Wright's stain and H and E was used for all specimens. Histopathological study was done whenever biopsy specimen was received and IHC study was employed whenever necessary.

Study Design- A cross-sectional study.

RESULTS

Among a total number of 1220 lymph node aspirates, adequate material was obtained in 1033 cases. The age range of the patients ranged from 6 months to 89 years and the male: female ratio was 1.43: 1. The cervical group of lymph nodes was the commonest site of involvement- 727 cases (70%). Benign lesions were seen in 802 patients (78%) and neoplastic lesions were seen in 231 patients (22%). Interpretation: Biopsies from the corresponding lymph nodes were received in 22 cases. Cytohistological correlation was found in 12 out of 22 cases (66.67%). Five cases of reactive lymphadenitis on cytology revealed tubercular lymphadenitis, non-Hodgkin's lymphoma (2), metastatic deposits and Castleman's disease. Accuracy for reactive lymphadenitis was 77.27%. Accuracy of cytological diagnosis of Hodgkin's and non-Hodgkin's lymphoma were found to be 100% and 77.27% respectively. Maximum number of correct diagnosis was obtained in metastatic carcinoma. Overall, the accuracy of Cytohistological correlation was found to be 14/22, i.e. 63.64%.

CONCLUSION

Aspiration cytology provides a reliable, safe, rapid and economic method of investigating lymph node enlargement. However, reactive lymphadenitis/ a negative cytology does not rule out serious underlying disorder. This should be considered keeping the overall clinical picture in mind. Also, the diagnostic accuracy can be further improved manifold when used in collaboration with other special techniques like cytochemistry, bacteriologic culture, immunocytochemistry, ultrastructural studies and molecular hybridisation.

KEYWORDS

Lymph Nodes, FNAC, Histological Correlation.

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BACKGROUND

Accurate diagnosis of lymphadenopathy is of utmost importance in planning proper therapeutic measures. This is possible with a multidimensional view of the problem facilitated by understanding of its clinical appearances,

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cytology, histology and other lab investigations before making a diagnosis. Even such an approach may fail to establish the aetiology in few cases. One of the most problematic areas in cytology is the distinction between a reactive and neoplastic lymphoid proliferation.¹

The Department of Pathology, KIMS, Hubli receives a good number of patients with lymphadenopathy for cytodiagnosis. Hence, an attempt is made to study the different varieties of lymphadenopathy, which will bear impact on patient management and prognosis.

Objectives

To evaluate the role of FNAC in diagnosis and management of lymphadenopathy- changing perspectives in view of better cytodiagnosis and fewer biopsies.

Study Design

A cross-sectional study.

MATERIALS AND METHODS

Source of Data

All patients presenting to the cytology section with lymphadenopathy and reference material submitted to the Department of Pathology, KIMS, Hubli for cytopathological study during the period from December 2010 to May 2012. FNAC was done with patient's verbal consent. Histopathological study of biopsy material of these cases was done wherever available.

Methods of Collection of Data

Clinical data was obtained from patient's examination, clinical records, lab investigations and requisition submitted for cytology of lymph nodes and tissue specimens received in the department for histopathological examination. The clinical and laboratory data of these patients were recorded on a structured proforma.

Inclusion Criteria

All patients presenting to the department with lymphadenopathy for FNAC study.

Exclusion Criteria

1. Cases where inadequate aspirate precludes diagnosis.
2. Cases under treatment for known primary or secondary neoplasm in lymph nodes.

Technique of FNAC^{2,3}

The aspiration procedure is simple and rapid, and inexpensively performed on both hospitalised and outpatients. Sedation or local anaesthesia is unnecessary. The largest and most prominent lymph node is selected. The selected lymph node is aspirated under strict aseptic precautions. Overlying skin is stretched, and the lymph node grasped between the index finger and thumb of left hand; a sterile 22 or 23 gauge needle is fitted to a 5 - 10 mL syringe and pierced obliquely into the lymph node. After entering the lymph node mass, the plunger is withdrawn, and the negative pressure created in the syringe. Then the needle is moved back and forth several times with a constant suction. The negative pressure is released, and the needle removed from the mass. Smears are prepared using another glass slide exerting light pressure.

Local lidocaine anaesthesia was used only when deep lesions were aspirated. A 20-cc glass syringe with an 18-gauge, 1.5-inch long needle was used. For deep-seated soft-tissue, transthoracic or transabdominal masses, 35-inch spinal needles were used.

Smears are immediately fixed in 95% ethyl alcohol and stained by Haematoxylin and eosin stain. Air-dried smears are also prepared and stained with Wright's stain. Other special stains used were Ziehl-Neelsen, Papanicolaou stain and GMS stain wherever necessary.

Histopathological study was done whenever biopsy specimen was received. IHC study, wherever necessary, was employed.

Ancillary studies like Mantoux test, haematological parameters and chest x-ray, serological tests for HIV, Brucella and bone marrow examination were done wherever necessary to arrive at a comprehensive diagnosis. In patients with source of infection, swab was taken for culture and sensitivity.

Ethical Clearance

Ethical clearance was obtained from Ethical Committee of our Institutes.

RESULTS

Total number of cases in the present study was 1220. Inadequate or no aspirates were obtained in 187. Adequate history and aspirate were obtained in 1033 cases. 85 patients were HIV positive.

Male: female patients were in the ratio of 6: 4. Incidence of malignant lesions was 23%.

Benign Lymphadenopathies

- Reactive lymphadenitis was the most common lesion in the cervical and axillary region followed by tubercular lymphadenitis.
- Reactive lymphadenitis was the most common lesion in inguinal region.

Malignant Lymphadenopathies

Metastatic squamous cell carcinoma was the most common lesion in cervical region. The second most common was metastatic adenocarcinoma deposits and poorly differentiated carcinoma deposits. We encountered 4 cases suggestive of Hodgkin's lymphoma and 6 cases of NHL. Other malignant lesions were deposits of papillary carcinoma thyroid, mucoepidermoid carcinoma and renal cell carcinoma.

Metastatic adenocarcinoma was the most common malignant lesion in the axillary region, most often the primary was from infiltrating ductal carcinoma of breast. Metastatic squamous cell carcinoma was the most common malignancy in the inguinal region.

Biopsies were obtained in 22 Cases. Table Cytohistological Correlation A

Sl. No.	Age	Sex	Cytology Diagnosis	Biopsy Diagnosis	Y/N	Final Diagnosis (IHC Etc.)
1.	55	F	REACTIVE LYMPHADENITIS	HODGKIN'S DISEASE	N	NHL- DLBCL
2.	45	F	REACTIVE LYMPHADENITIS	CASTLEMAN'S DISEASE	Y	
3.	30	M	POORLY DIFF CA DEPOSITS	NHL- SMALL CLEAVED CELL TYPE	N	
4.	65	F	ADENOCARCINOMA DEPOSITS	ADENOCARCINOMA DEPOSITS	Y	
5.	25	F	NHL	DSRCT	N	DSRCT
6.	40	F	PAPILLARY CA THYROID	PAPILLARY CA THYROID	Y	
7.	35	F	PAPILLARY CA THYROID	PAPILLARY CA THYROID	Y	
8.	60	M	POSSIBILITY OF NHL TO BE CONSIDERED	CASEATING TUBERCULAR LN	N	
9.	35	F	GRANULOMATOUS LYMPHADENITIS	GRANULOMATOUS LYMPHADENITIS	Y	
10.	45	F	REACTIVE LYMPHADENITIS	REACTIVE LYMPHADENITIS	Y	
11.	65	F	METASTATIC SQ CELL CARCINOMA	METASTATIC SQ CELL CARCINOMA	Y	
12.	45	F	REACTIVE LYMPHADENITIS	REACTIVE LYMPHADENITIS	N	SMALL LYMPHOCYTIC LYMPHOMA
13.	45	M	REACTIVE LYMPHADENITIS	REACTIVE LYMPHADENITIS	Y	
14.	50	M	REACTIVE LYMPHADENITIS	REACTIVE LYMPHADENITIS	Y	
15.	18	M	HODGKIN'S LYMPHOMA	HODGKIN'S LYMPHOMA	Y	
16.	40	M	NHL	NHL	Y	NHL- LOW-GRADE B-CELL TYPE

17.	47	M	CASEATING TUBERCULAR LN	CASEATING TUBERCULAR LN	Y	
18.	53	M	REACTIVE LYMPHADENITIS	TUBERCULAR LYMPHADENITIS	N	
19.	58	M	REACTIVE LYMPHADENITIS	MODERATELY DIFFERENTIATED SQUAMOUS CELL CA	N	
20.	50	F	REACTIVE LYMPHADENITIS	REACTIVE LYMPHADENITIS	Y	
21.	52	F	POORLY DIFFERENTIATED CARCINOMA DEPOSITS	METASTATIC UNDIFFERENTIATED CARCINOMA- PROBABLY NASOPHARYNGEAL	Y	METASTATIC UNDIFFERENTIATED CARCINOMA- UPPER AERODIGESTIVE TRACT OR SALIVARY DUCT CA
22.	17	M	NHL	NHL	Y	

Table Overall Cytohistological Correlation

Cytological Diagnosis	Histological Diagnosis	
	Positive	Negative
Positive	14	2
Negative	4	2

Sensitivity= $14/18 = 77.78\%$

Specificity= $2/4 = 50\%$

PPV= $14/16 = 87.5\%$

NPV= $2/6 = 33.34\%$

Accuracy= $14/22 = 63.64\%$

Overall the Accuracy of Cytohistological Correlation was found to be 14/22, i.e. 63.64%

Reactive Lymphadenitis/Cytohistological Correlation

Four cases of reactive lymphadenitis, diagnosed on histopathology were also diagnosed on cytology.

5 cases reported as reactive on cytology showed features of tubercular lymphadenitis, Hodgkin's lymphoma, non-Hodgkin's lymphoma, metastatic deposits of squamous cell carcinoma and Castleman's disease (one each) on histological examination. The case diagnosed as Hodgkin's lymphoma-mixed cellularity was diagnosed as diffuse large B-Cell lymphoma on IHC.

Calculations for Reactive Lymphadenitis

	Histopathology Positive	Histopathology Negative	
Cytology Positive	4	5	PPV= $a/a+b$
Cytology Negative	0	13	NPV= $d/c+d$
	Sensitivity= $a/a+c$	Specificity= $d/b+d$	Accuracy= $a+d/a+b+c+d$

Therefore, the sensitivity= 100%

Specificity= 72.2%

Accuracy= 77.27%

Tubercular Lymphadenitis/ Cytohistological Correlation

Two cases of tubercular lymphadenitis diagnosed on histopathology were also diagnosed on cytology.

An additional 2 cases were missed on cytology as reactive lymphadenitis and non-Hodgkin's lymphoma.

Calculations for Tubercular Lymphadenitis

	Histopathology Positive	Histopathology Negative	
Cytology Positive	2	0	PPV= $a/a+b$
Cytology Negative	2	18	NPV= $d/c+d$
	Sensitivity= $a/a+c$	Specificity= $d/b+d$	Accuracy= $a+d/a+b+c+d$

Therefore, the Sensitivity= 50%

Specificity= 100%

Accuracy= 90.91%

Hodgkin's Lymphoma/ Cytohistological Correlation

One case of Hodgkin's lymphoma diagnosed on histopathology was diagnosed on cytology.

Calculations for Hodgkin's Lymphoma

	Histopathology Positive	Histopathology Negative	
Cytology Positive	1 a	0 b	PPV= $a/a+b$
Cytology Negative	0 c	21 d	NPV= $d/c+d$
	Sensitivity= $a/a+c$	Specificity= $d/b+d$	Accuracy= $a+d/a+b+c+d$

Therefore, the Sensitivity= 100%

Specificity= 100%

Accuracy= 100%

Non-Hodgkin's Lymphoma/ Cytohistological Correlation

Four cases of NHL diagnosed on histopathology were also diagnosed on cytology.

Three cases were missed on cytology as reactive lymphadenitis (2) and deposits of poorly differentiated carcinoma (1). Additional 2 cases reported as NHL on cytology showed features of tubercular lymphadenitis and Desmoplastic Small Round Cell Tumour on histological examination. One of the four cases diagnosed as non-

Hodgkin's lymphoma was confirmed on IHC as NHL low-grade B cell type.

Calculations for Non-Hodgkin's Lymphoma

	Histopathology Positive	Histopathology Negative	
Cytology Positive	2	2	PPV= a/a+b
Cytology Negative	3	15	NPV= d/c+d
	Sensitivity= a/a+c	Specificity= [d/b+d]	Accuracy= a+d/a+b+c+d

Therefore, the Sensitivity= 40%

Specificity= 88.235%

Accuracy= 77.27%

Metastatic Carcinoma Deposits/ Cytohistological Correlation

Among them, one patient was positive for HIV. One case of deposits of squamous cell carcinoma was diagnosed on cytology and one was missed.

Cytohistological Correlation

Two cases had lymph node excised, among which one was diagnosed as NHL and other was diagnosed as metastatic deposits of undifferentiated carcinoma from upper aerodigestive tract.

Calculations for Metastatic Deposits in Lymph Node

	Histopathology Positive	Histopathology Negative	
Cytology positive	5	1	PPV= a/a+b
Cytology negative	1	15	NPV= d/ c+d
	Sensitivity= a/a+c	Specificity= [d/b+d]	Accuracy= a+d/a+b+c+d

Sensitivity= 5/ (5+1) = 83.33%

Specificity= 15/ (1+15) = 93.75%

Accuracy= 5+15/ 22= 90.90%

Therefore, overall for Diagnosis of Metastatic Deposits

- Sensitivity= 83.33%
- Specificity= 93.75%
- Accuracy= 90.90%

DISCUSSION

Fine needle aspiration cytology is a useful adjunct to conventional method of diagnosis in cases of mass lesions. It is a simple, cost effective, safe and time saving procedure. Lymphadenopathy is one of the common clinical presentations of various ongoing disease processes inside the body. The aim of the present study was to evaluate the role of FNAC in cases of lymphadenopathy of various aetiologies, to find the frequency and cause of lymph node enlargement in patients of different age groups and analyse the different cytomorphological patterns associated with various lymphadenopathies.

The results in this study show that fine needle aspiration cytology is a useful adjunct to clinical diagnosis for triage of patients into those who require biopsy. Fine needle aspiration cytology was performed in 1220 cases of lymphadenopathy, both superficial and deep.

The lesions arising in the lymph node can be found in patients ranging from early to advanced age. In our study, the youngest patient was 6 months old, male, HIV positive with reactive lymphadenitis. The oldest was 89 years old female with metastatic squamous cell carcinoma deposits in cervical lymph node from primary in the cheek. These figures come in close comparison to other studies.^{4,5}

The male preponderance (59%) in our series correlates well with studies done by Agarwal⁴ (57.2%), whereas male-to-female ratio was equal in other series by Hag et al.⁶ As regards to the site of FNAC, cervical region (70%) was the commonest site of lymphadenopathy followed by axillary region (15%) as observed by Ahamad SS et al,⁷ Hirachand et al⁸ and Agarwal et al.⁴

Comparison of Distribution in Different Regions in Different Studies

Region	Agarwal et al ⁴	Ahamad SS et al ⁷	Hirachand et al ⁸	Present Study
Cervical	91.1%	73.5%	75.4%	70%
Axillary	5.78%	10.6%	15.4%	15%
Inguinal	---		9.2%	9%
Other/ Multiple	3.12%	15.9%	--	6%

Out of total 1033 cases, 820 cases (78%) were benign and 23 (22%) were malignant lesions.

Comparison of Diagnosis in Various Studies

Cytologic Diagnosis	Ahamad SS et al ⁷	Hirachand et al ⁸	Agarwal et al ⁴	Present Study
Benign	86.4%	81.7%	80.29%	78%
Reactive	53.6%	41.5%	42.15%	47.53%
Tubercular	32.8%	28%	27.19%	20.53%
Malignant	13.6%	18.3%	19.71%	22%
Primary	4.5%	6%	2.1%	1.74%
Metastasis	9.1%	12.3%	17.6%	20.36%

Various causes have been found to be responsible for lymphadenopathy and their frequency varies according to the age of the patient. Out of the total 1033 patients, the most common aetiological factor in lymphadenopathy was reactive lymphadenitis followed by tubercular lymphadenitis which is comparable with studies done by Aggarwal et al,⁴ Ahmad et al⁷ and Hirachand et al.⁸

As in the present study, Hirachand et al⁸ and Hadju et al⁹ also noted that the commonest type of metastatic carcinoma to lymph node was of squamous cell variety (9.2%) followed by adenocarcinoma (6.7%). We encountered a lower incidence of lymphoma/ primary malignancies of lymph nodes compared to other studies, probably because ours is a tertiary care institute and not a referral centre for cancer.^{4,7,8}

The most common tumour metastases to the neck nodes was the squamous cell carcinoma (10.6%) arising commonly in the cheek, tongue, buccal mucosa, alveolus, palate and

nasopharynx followed by adenocarcinoma (3.7%) and poorly differentiated carcinoma deposits (3.7%).

In the axillary region, metastatic adenocarcinoma from primary in the breast was the most common malignant lesion (22.4%) similar to that noted by Gupta et al.¹⁰

In the non-Hodgkin's lymphoma group, 2 cases were of leukaemic infiltration in the lymph node. In such cases, it is very difficult to differentiate between non-Hodgkin's lymphoma and leukaemic infiltration on examination of cytological smears alone as noted by Pandit et al.¹¹

Comparison of Number of Biopsies in our Institute a Decade Ago

Time period	2000 - 02	2010 - 12
Number of lymph node biopsies received in the department	311	22

Above table highlights the marked reduction in the number of lymph node biopsies performed at our hospital a decade after increased application of FNA.

Cytohistological correlation was possible in 22 cases from the same lymph node.

Maximum number of correct diagnoses was obtained in metastatic carcinoma (5 cases) as seen by Pandit et al¹¹ and Narang et al.¹²

In the present series, there were 4 cases of tubercular lymphadenitis diagnosed by histopathology. Two were correctly diagnosed by FNAC. In the remaining two, one was missed as reactive hyperplasia and one as non-Hodgkin's lymphoma.

Out of the 18 cases of lymphoma, biopsy was received in five and diagnosis was confirmed in three cases. Diagnostic accuracy was observed to be 77.27% in these cases. Ruchi et al,¹³ Gupta et al¹⁴ and Tripathi et al¹⁵ reported it as 84%, 82% and 80% respectively in their study of lymphoma cases. We have found it difficult in two cases to differentiate reactive hyperplasia from lymphoma. The possibility of overlap among reactive hyperplasia, non-Hodgkin's lymphoma and Hodgkin's is also mentioned by Gupta et al¹⁴ who exclusively studied aspiration smears of lymphoma cases.

One of the most problematic areas in cytology is the distinction between a reactive and neoplastic lymphoid proliferation.¹ The diagnosis of a malignant lymphoma is straightforward when bizarre or highly pleomorphic lymphoid cells are present in the aspiration smears.¹ Otherwise, the cytologic diagnosis of malignant lymphomas often is based on the presence of a relatively monomorphic lymphoid population, whereas a polymorphous lymphoid population typically is observed in reactive lymphoid processes.¹ Potential misdiagnoses may occur when certain lymphomas present with an apparently heterogeneous cellular pattern.¹

Differentiating HL from NHL is particularly important, since it has direct implications for the prognosis and treatment of patients.¹⁶ The histologic pictures of their classic forms are sufficiently characteristic to allow for confident differential diagnosis in most cases.¹⁶ Nonetheless, it was noted that 20% to 30% of patients with HL treated with the otherwise successful standard therapy do not respond to treatment and finally die from their disease.¹⁶ The new

methodologies allowing for immunophenotypic and genetic analysis of the neoplasms revealed the existence of lymphomas that did not fit exactly in the categories previously defined, because of a variety of overlapping features.¹⁶ Such cases with mixed features, a few of which had been reported since the early 1990s were the subject of two workshops and several studies dedicated to these entities, presently referred to as gray-zone lymphomas.¹⁶

Lymphomas that exhibit features overlapping with those of the classical type of HL belong to three groups: (a) Nodular lymphocyte-predominant HL (NLP HL) and T-cell rich large B-cell lymphoma (TCRLBCL); (b) Anaplastic large-cell lymphomas (ALCL; ALK+ and ALK-) and peripheral T-cell lymphoma (PTCL); and (c) Primary mediastinal large-cell B-cell lymphoma (PMLBCL) and diffuse large B-cell lymphoma (DLBCL).¹⁶

1. In NLP HL, the neoplastic cells (L and H cells) are of B-cell type; however, they are clearly different from H/R-S cells of classical HL.¹⁶
2. T-cell rich large B-cell lymphoma may show cells of H/R-S type, but they are of B-cell type and express BCL-2. Eosinophils are not in the background, as in classical or HL, and EBV gene products are not identified.¹⁶
3. The cells of ALCL tumours, even when similar to H/R-S cells commonly express the T-cell antigens and in most cases the ALK fusion gene which is always negative in HL. The ALK-ALCL lymphomas can be distinguished by the lack of PAX5 antigen, which is usually dimly positive in H/R-S cells.¹⁶
4. Peripheral T-cell lymphomas express T-cell markers as well as cytotoxic molecules such as granzyme B, whereas HLs are of B-cell origin and do not express T-cell or cytotoxic markers.¹⁶
5. The third group of large B-cell lymphomas constitute the most common overlapping gray zone with HLs, since it is now established that the latter also originate in the B-cells of the lymphoid follicles.¹⁶ The large B-cell lymphomas arising primarily in the mediastinum originate in the B cells of the thymic medulla and by mediastinal location and histologic similarity may create difficult problems of differential diagnosis with HL.¹⁶ These gray-zone lymphomas share features of DLBCL and of classical HL with expression of CD30, CD15 as well as the pan-B-cell antigens CD20 and CD79a suggesting that they represent a transition between the two tumour types.¹⁶

Gray-zone lymphomas generally exhibit a more aggressive behaviour than their HL counterparts, thus underlining the importance of their differential diagnosis.¹⁶ They require distinct treatments and the addition of the anti-B-cell rituximab antibody to the full combination chemotherapy has been suggested.¹⁶

CONCLUSION

Aspiration cytology provides a reliable, safe, rapid and economic method of investigating lymph node enlargement. However, reactive lymphadenitis/ a negative cytology does not rule out serious underlying disorder. This should be considered keeping the overall clinical picture in mind. Also, the diagnostic accuracy can be further improved manifold when used in collaboration with other special techniques like

cytochemistry, bacteriologic culture, immunocytochemistry, ultrastructural studies and molecular hybridisation.

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