

Profiling the Cytopathological Diagnosis of Enlarged Lymph Nodes on Fine Needle Aspiration Cytology

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ABSTRACT

Background: This study aimed to assess the distribution of fine-needle-aspiration-cytology or biopsy findings through cytopathological patterns of lymph nodes aspirations in different age groups of the population visiting a tertiary level hospital in Kathmandu.

Methods: The retrospective study was conducted on the database of the 226 lymphadenopathy cases that underwent biopsy fine needle aspiration cytology from 1 January 2016 to 31 December 2019 at the Department of pathology of Helping Hand's Community Hospital, Kathmandu. A descriptive analysis was performed yielding proportions and counts for the quantitative variables. Chi-squared test was used to compare the proportions. Point estimates and confidence intervals for measures of association were assessed with 95% confidence interval and p-value ≤ 0.05 was considered statistically significant.

Results: The 20-40 years were the most common age group undergoing biopsy fine needle aspiration cytology procedures. Males recorded majority of cases (68%, 153 of 226). The highest incidence of Reactive lymphadenitis was obtained (50%, 113 of 226) and it was the most common findings in the age group of <20 and 40-60 years. The Cervical lymph node (86%) was the most common anatomical location followed by axillary lymph nodes (8%). Squamous cell carcinoma and Adenocarcinoma accounted for more than half (53%, 20 of 38) of all the diagnosed malignancy. The incidence of Non-Hodgkin lymphoma and Hodgkin lymphoma were 8% ($n = 3$ of 38) and 5% ($n = 2$ of 38) respectively.

Conclusions: Lymph node enlargement is associated with a wide range of etiologies with Reactive lymphadenitis as the most common cause and cervical lymph node as the common site.

Keywords: Cytopathology; fine needle aspiration cytology; lymph node.

INTRODUCTION

Fine-needle aspiration cytology (FNAC) is widely recognized as a safe, minimally invasive, and cost-effective method of assessing lymph node abnormalities.^{1,2} Despite several etiologies for lymph nodes enlargement, FNAC is useful in preoperative evaluation of the etiology, to determine whether it is infectious, inflammatory, or neoplastic and to stage the malignancy or to monitor recurrences as well as the progression of malignancy.^{3,4}

Ancillary tests like immunocytochemistry, flow cytometry, fluorescence in situ hybridization have revolutionize interpreting cytologies correctly.⁵ However, there has been little progress of auxillary testing methods in Nepal. As the disease process is continuously evolving, it is important to understand the

variation of cytologic patterns of diseases in the country to improve the diagnosis. The objective of the study was to assess the distribution of FNAC findings through cytopathological patterns of lymph nodes aspirations in different age groups of population visiting the hospital in Kathmandu.

METHODS

A retrospective study was conducted on the data of the 226 lymphadenopathy cases that underwent FNAC between January 2016 and December 2019 at Department of Pathology of Helping Hand's Community Hospital, Kathmandu. The database was obtained in Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) files. The information of study variables on age, sex, clinical examination of the anatomical location and swellings were obtained. The cases signed out as

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inadequate for the evaluation such as smears having blood only, or lacking any cells were excluded from the study. The diagnosis was grouped into epidemiological and cytopathological parameters to identify an association between patient age and sex.

A sample of cells and tissue materials for FNAC were obtained using a 10-milliliter syringe attached to a 20 to 24-gauge needle that provided a closed system to apply negative pressure for aspiration. The aspirated material was expelled on the glass slides to prepare multiple smears. The number of slides prepared was decided on the quantity of material, the diagnostic needs. The slides were air-dried for Romanowsky staining and alcohol-fixed for Papanicolaou staining. Ziehl Neelsen stain was also utilized depending on the clinical scenario where tubercular etiology was suspected. Smearing was prepared rapidly by pulling apart two slides with the aspirated material in between applying gentle but firm pressure. Air-dried slides, as well as alcohol wet slides were prepared for the procedure.

An exploratory data analysis was performed yielding proportions and counts for the quantitative variables using contributed package epiR⁶ in the program R.⁷ Chi-squared (x2) test was used to compare the proportions. Point estimates and confidence intervals for measures of association were assessed with 95% confidence interval and P-value ≤0.05 was considered statistically significant.

An ethical approval was obtained from the Ethical Review Board of Nepal Health Research Council (Registration number: 424/2021) and the Ethical Committee for Clinical Research of the Helping Hand’s Community Hospital.

RESULTS

In the study period, 226 cases were identified that underwent FNAC of which 153 (68%) were males. The

number of FNAC identified cases were divided arbitrarily into four different age groups: <20 years, 20-40 years, 41-60 years, and >60 years (Table 1) of which the age group 20-40 recorded most frequent cases (84 of 226, 37%, 95% CI 31 to 44) of lymphadenitis. The age group <20 years were 2.05 times more likely to have Reactive lymphadenitis than other age groups (prevalence ratio 2.05; 95% CI 1.62 to 2.60; p <0.001).

Of 226 cases, the most common site for FNAC procedure was the cervical lymph node (86%) followed by axillary lymph nodes (8%), inguinal lymph nodes (5%), and intra-abdominal mesenteric lymph nodes (1%). Ninety-three percent of the cases were carried out by FNAC technique without any imaging-guided assistance whenever the lymph nodes were superficial and clinically palpable. Ultrasonogram guided FNAC was performed in 6% of deep-seated cervical lymph node cases which were difficult to palpate and 1% of intra-abdominal mesenteric lymph nodes cases.

Reactive lymphadenitis (n = 113) was the predominant findings indicating the odds of Reactive lymphadenitis for the age group (41 - 60) was 1.30 (95% CI 0.64 - 2.67) times that of other age groups. Reactive lymphadenitis is characterized by enlarged lymph nodes cluster exhibiting a polymorphous lymphoid population of cells suggestive of various phases of development, with occasional tingible body macrophages on cytologic studies without any abnormal cells or morphological alterations such as granulomas development or necrosis. The diagnosis of Tubercular lymphadenitis was made in 23% (n = 51 of 226) cases when Acid Fast Bacilli (AFB) was detected after Ziehl Neelsen stain. The presence of bacilli was almost always accompanied by necrotic substances.

The epithelioid cell granulomas, Langhans giant cells were also present in variable proportions with or without necrosis in the Tubercular lymphadenitis cases. Incidence of malignant causes was most frequent

Table 1. Cytological findings by FNAC stratified by different age groups (n = 226).

Age group (years)	Type of lymphadenitis					Total cases (n)	% (95% CI)
	Reactive	Granulomatous	Suppurative	Tubercular	Malignancy		
<20	53	2	9	4	-	68	30 (24, 36)
20-40	36	5	1	39	3	84	37 (31, 44)
41-60	20	6	-	8	2	36	16 (12, 21)
>60	4	-	1	-	33	38	17 (13, 22)
Total	113	13	11	51	38	226	100

Abbreviation: CI = Confidence Interval

in the elderly age group 60 above. Of 226 cases, the Granulomatous lymphadenitis was observed in 5.7% ($n = 13$).

Table 2. Types of malignant findings identified by FNAC ($n = 38$).

Malignancy types	Number of cases	
	<i>n</i>	%
Adenocarcinoma	12	31.58
Squamous cell carcinoma	8	21.05
Undifferentiated carcinoma	7	18.42
Non-Hodgkin lymphoma	3	7.89
Papillary carcinoma thyroid	3	7.89
Small cell carcinoma	2	5.26
Hodgkin lymphoma	2	5.26
Melanoma	1	2.63
Total	38	100

Thirty-eight cases showed different types of malignancy nature with Adenocarcinoma accounting for the most prevalent (Table 2). People of older age (>60 years) were more likely to have malignancy compared to other age groups (χ^2 test statistic 160.159; $df = 1$; $p < 0.001$). Squamous cell (Figure 2) carcinoma and Adenocarcinoma accounted for more than half (53%, $n = 20$ of 38) of all the diagnosed malignancy.

The 8% ($n = 3$ of 38) cases of lymph node aspirate showed diagnostic characteristics of Non-Hodgkin lymphoma with monotonous population of atypical lymphoid cells in the smear. While the cytologic result of 5% ($n = 2$ of 38) Hodgkin lymphoma showed large malignant-looking lymphoid cells or typical Reed-Sternberg cells.

DISCUSSION

This study presented a spectrum of pathological findings responsible for lymph node enlargement due to benign reactive process, infective or malignant causes. Early consideration of biopsy using FNAC is crucial in identifying potential underlying disease through cytomorphological information whenever there is lymphoproliferative cases or high levels of serum sIL-2r or lactate dehydrogenase.^{8,9}

Previous study found that reactive lymphadenitis as the most common cause of lymphadenopathy. The problem with reactive lymphadenitis cases were the obscure etiology associated with it which were hard to determine.¹⁰ It was consistent with this study findings where cases of reactive lymphadenitis were the most

frequent cause of lymphadenopathy. Similar findings were reported in a study where 42.5% of cases were Reactive lymphadenitis, 12.3% were metastatic and 6% of cases were lymphomas.² In contrary, another study reported 23.5% Reactive lymphadenitis in the lymph node aspirates.¹¹ Zhou *et al* evaluated 53.6% cases as malignant and 2.9% Tuberculosis.¹²

Studies have shown tubercular lymphadenitis as the most frequently identified lesion (48%, $n = 151$ of 310), followed by 32% ($n = 102$ of 310) granulomatous lymphadenitis.¹³ Similar to our study, the same study showed the Cervical lymph nodes to be the most common group of lymph nodes to undergo FNAC followed by an axillary group.¹³

The nature of aspiration obtained in the study was fluid, purulent, or hemorrhagic. The main causes of purulent aspirates from lymph nodes are tubercular lymphadenopathy, malignancies, cat scratch disease and many bacteriological conditions. The nature of aspirates indicating Granulomatous lymphadenitis varies according to its infectious or non-infectious origin.^{14, 15} Infectious causes include suppurative and non-suppurative conditions. The causes of suppurative granulomatous disorders include cat scratch disease, Yersinia infection, Tularemia. Non-suppurative granulomatous disorders include tuberculosis, toxoplasmosis, brucellosis, and syphilis. The non-infectious granulomatous disorder includes sarcoidosis. Further histopathological, microbiological and biochemical tests are required to identify the etiological agent for granulomatous lymphadenitis.

This study findings showed that the presence of necrosis was associated with increased detectability of tubercular bacilli, which is similar to the findings by Das *et al*.¹⁶ The same study reported that cases with lymphocytes, epithelioid histiocytes, and multinucleated giant cells are associated with less likelihood of AFB (TB) detection. In this study, 51 cases (22.5%) showed positivity to Ziehl Neelsen stain confirming the case of tuberculous lymphadenitis. Another study reported Tubercular lymphadenitis as the most common cause of lymphadenopathy with 61.8% showing AFB positivity.¹⁷ The study also showed that Auramine Rhodamine positivity with the help of fluorescent microscopy was a better diagnostic tool than Ziehl Neelsen staining to detect AFB.

A considerable proportion (18%) of malignant diagnoses were attributed to metastatic causes and 1% of cases were due to lymphomas.¹⁸ Several studies have linked enlarged solitary lymph nodes and Metastatic carcinoma with older age than in children.^{19, 20} Consistent with the

literature, this study found that older age (>60 years) were more likely to have malignancy than younger (prevalence ratio 32.65; 95% CI 13.63 to 78.22; $p < 0.001$). It has been suggested that the chances of malignancy as a cause of peripheral lymphadenopathy increases over the age of 40 years.²¹ Enlarged lymph nodes in the posterior cervical triangle and the supraclavicular areas are more likely to be due to malignancy than enlarged lymph nodes in other areas.⁵ In this study, only Hodgkin lymphoma and metastatic papillary carcinoma thyroid occurred in the younger group, the rest of the cases of malignancy lymphadenopathy exclusively occurred in age group of >60 years.

This study identified Adenocarcinoma as the most common cause of metastases in the lymph node. In contrast, studies have reported squamous cell carcinoma as the most common cause of metastases to the lymph node.²²⁻²⁴ Interestingly, two cases of metastatic papillary carcinoma thyroid were observed in relatively younger age group of 20-40 years and single case in the age group of 40-60 in this study.

The FNAC itself has its own limitations to make specific diagnosis. FNAC requires an experienced operator and it has been advised that FNAC of lymph node be carried out with ROSE (Rapid On Site Evaluation) to evaluate the adequacy of the collected material and for triaging. Another shortcoming of FNAC is the difficulty in differentiating benign from malignant primary salivary gland neoplasm and requires extreme caution.²⁵ Most of the time, the cause of reactive lymphadenopathy remains obscure. Surgical excision biopsy evaluation is indicated in this condition when clinical and imaging findings show marked disparity or lymph node enlargement persists for a prolonged time. The availability of immunophenotyping has been instrumental in the diagnosis and typing of lymphoma distinguishing the origin of a malignant tumor that has metastasized to the lymph node.¹² Ancillary studies like flow cytometry, cytogenetics, immunocytochemistry, molecular studies, microbiological studies for an infectious process, serology are utilized from the sample obtained by FNAC to arrive at the definitive diagnosis.

The importance of information regarding the patient's age, gender, history of present illness, the nature and amount of aspirate, site of aspirate along with reliable imaging findings cannot be exaggerated while reporting any FNAC case. Since reporting of FNAC depends on the interpretation of cell morphology on the smear which is obtained following the procedure, it has its limitations. For example if a lymph node is just partially involved

and the needle doesn't happen to hit the specific area, the aspirate is going to be devoid of those cells from the affected areas, resulting in misinterpretation of the diagnosis. Such cases of partially affected lymph node are not uncommon in cases of Hodgkin lymphoma and metastases.

Completely necrotic lymph nodes due to infectious or malignant causes pose another challenge, since the lack of viable cells hampers meaningful interpretation. A similar scenario occurs when the lymph node has cystic changes and the aspirate shows fluid material lacking any substantial numbers of cells. Such scenarios could prove to be a red herring for the examining pathologist. Differential diagnoses included in such cases are lymphoepithelial cyst, warthin tumour, metastases from squamous cell carcinoma, or papillary carcinoma thyroid.

CONCLUSIONS

Lymph node enlargement is associated with a wide range of etiologies with Reactive lymphadenitis as the most common cause and cervical lymph node as the common site.

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