Research Article

Cytological spectrum of lymph node lesions-our institute experience

Patil R. K.[1], Anubrolu I. P.[2], Kittur S. K.[3], Haravi R. M.[4], Aruna S.[5], Jadhav M. N.[6]

[1]Dr. Patil Rashmi K., Associate Professor, [2]Dr. Indira Priyadarshini A, Post Graduate, [3]Dr. Kittur Shreekant K., Professor and Head, [4]Dr. Haravi Rekha M., Associate Professor, [5]Dr. Aruna S., Associate Professor, [6]Dr. Jadhav Meena N., Associate Professor, all authors are affiliated with Department of Pathology, Belagavi Institute of Medical Sciences, Belagavi, Karnataka, India.

Address for Correspondence: Dr. Indira Priyadarshini A., Post graduate Student, Department of Pathology, Belagavi Institute of Medical Sciences, Belagavi. Karnataka, India, Email: indiraanubrolu@gmail.com

Abstract

Introduction: Lymphadenopathy is of great clinical significance as underlying diseases may range from a treatable infectious etiology to malignant neoplasms. Fine needle aspiration cytology (FNAC) plays a vital role in solving these issues, being recognised as rapid diagnostic technique because of simplicity accuracy and minimal invasion. **Aim**: To know the cytological spectrum of lymphadenopathy and to understand the burden of disease in this region. **Design:** This is a retrospective cytological study on 1478 patients including all age groups and both genders presenting with palpable lymphadenopathy over a period of seven years. **Materials and Methods:** FNAC was conducted with 22-24 gauge needle attached to 20 cc syringes. Smears were stained with Giemsa and Papanicolaou stains routinely; and special stains like Zeihl-Neelsen and periodic acid-Schiff were done whenever required. The data were tabulated as per the involvement of various lymph node groups and type of neoplastic and non-neoplastic lesions involved. Data was analysed using SPSS 22 version. **Results:** Out of 1478 aspirations of lymph nodes, the most frequent cause of lymphadenopathy was found to be tuberculous lymphadenitis 592 (40.06%) cases. The next frequent diagnosis was reactive lymphadenopathy with 550 (37.2%) cases, followed by metastatic lymphadenopathy in 243 (16.4%) cases. The diagnosis of primary neoplastic lesions was seen in 29 (2.0%) cases. **Conclusion:** In our study, the predominant cause of lymphadenopathy was tuberculous lymphadenitis, followed by reactive lymphadenopathy and malignant neoplasms especially metastatic carcinoma. FNAC was helpful in diagnosing approximately 98% of cases.

Keywords: FNAC, Lymphadenopathy, Metastatic lymphadenopathy, Tuberculous lymphadenitis

Introduction

Lymphadenopathy is a commonly encountered clinical problem which has multitude of causes varying from non-neoplastic to neoplastic conditions like inflammation, infection, primary or metastatic tumors [1]. Enlarged lymph nodes were the first organs to be sampled by fine needle aspiration [2]. Fine needle aspiration cytology (FNAC) has an important diagnostic role in the evaluation of peripheral lymphadenopathy and also helps in deciding the appropriate management [3]. It is very simple, reliable, cost effective and safe alternative to excision biopsy [4]. In our study, we have studied non-neoplastic and neoplastic lesions of enlarged lymph nodes by FNAC in patients presenting with peripheral lymphadenopathy to determine the pattern of disease affecting lymph nodes in our region.

Our study highlights the epidemiological and cyto-morphological spectrum of lymph node lesions and diagnostic utility of FNAC in arriving at early diagnosis and prompt treatment. As there are few studies of FNAC of spectrum of lymphadenopathy in North Karnataka region we have undertaken this study to reveal the burden of the disease in our institute.

Materials &methods

In this retrospective study, we have included patients of all age groups and both genders who gave consent for FNAC.

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Study design: This observational study was undertaken after obtaining approval from Institutional Ethics Committee.

Setting: Study was done in the cytology section, department of pathology of Belgaum Institute of Medical Sciences, Belgaum for seven years from 2009 to 2015.

Inclusion criteria: Patients of all age groups and both genders who underwent FNAC of enlarged lymph node were included in the study.

Exclusion criteria: FNAC cases with inadequate material were excluded from the study.

Variables: Age, sex, anatomical group of lymph nodes, laterality, single or multiple groups of lymph nodes and spectrum of lesions were taken into consideration.

Data source: Data was retrieved from the requisition forms in cytology section of department of pathology and the slides werereviewed for all cases during the period from 2009 to 2015 and included 1478 cases.

Sample size: The minimum number of cases required for the study was 1164 cases and 1478 cases were included in the study that was calculated by referring the article by Sharma et al[4] using the formula-n= z^2 x p x q / d^2; where z= z value for alpha error i.e., 1.96, p=proportion of tubercular lymphadenitis from the reference article=56.92, q=100-p=43.08 and d=5% relative error.

Statistical methods: Basic data was presented in percentage and diagrams wherever necessary. Chi-square test was used 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.

The FNAC smears were stained by Giemsa and Papanicolaou (Pap) stains routinely; and special stains like Zeihl-Neelsen (ZN) and periodic acid-Schiff (PAS) were done as and when required. The clinico-cytological features of all cases were recorded. The results were categorised into non-neoplastic and neoplastic lesions, which were further divided into various lesions.

Results

A total of 1478 FNAC cases with palpable lymph nodes were studied. The age of the patients ranged from first year of life to 98 years with mean age of presentation at 34 years in males and 31 years in females for all lymph node lesions. The youngest patient was a case of reactive lymphadenopathy and the oldest patient was a case of metastatic squamous cell carcinoma. The maximum number of patients 299 (20.23%) were in the age group of 21-30 years followed by 293 (19.82%) in 31-40 years and 39 (2.64%) cases in the age group of above 70 years (Table 1). In this study, slight male predominance was seen with male: female ratio of 1.2:1. Male patients constituted 811 (55%) and female patients were 667 (45%) out of 1478 patients. Upon correlation with age-wise and sex-wise distribution of cases, males were commonly affected in the age group of 31-40 years (151 cases) and females were affected a decade earlier in the age group of 21-30 years (180 cases). The findings had statistical significance of *P*<0.001. All types of lesions were common in males except for tuberculous lymphadenitis, which was common in females.

Table-1: Distribution of cases according to age & sex

AGE	Males	Females	Total
<10	144	91	235
11-20	115	98	213
21-30	119	**180**	299
31-40	**151**	142	293
41-50	98	68	166
51-60	87	42	129
61-70	68	36	104
>70	29	10	39
Total	**811 (55%)**	**667 (45%)**	**1478**

Out of various anatomical groups of lymph nodes, cervical lymph nodes were the commonest group to be affected in all the types of lymph node lesions. This showed a statistical significance of $P< 0.001$. Cervical lymph nodes were enlarged in 1020 of 1478 (69.0%) cases, followed by axillary lymph nodes in 112 (7.6%) cases. Other lymph node groups included submandibular 94 (6.4%), inguinal 71 (4.8%), supraclavicular lymph nodes 72 (4.8%), and few less common sites 109 (7.4%) like: submental, suprasternal,pre- and postauricular, occipital, epitroclear, suprascapular, inframammary, infraclavicular and epigastric lymph nodes. The lymph node groups on right side (772 cases) were commonly affected compared to left side (600 cases), and 34 lymph nodes were bilateral, in 41 cases lesions were seen in midline group and 31 cases presented with multiple anatomical site involvement of lymph nodes.

The spectrum of lymph node lesions studied by FNAC were divided into non-neoplastic and neoplastic lesions. The non-neoplastic lesions constituted majority of cases seen in 1206 (81.60%) patients as compared to neoplatic lesions seen in 272 (18.40%) cases. In non-neoplastic lesions, tuberculous lymphadenitis was the most frequent diagnosis with 592 (40.06%) cases, followed by other lesions (Table 2). Tuberculous lymphadenitis cases were further categorised based on cytological patterns into four groups (Figure 1): Group 1: Epithelioid granulomas without caseous necrosis- 241/592; Group 2: Epithelioid granulomas with caseous necrosis- 270/592; Group 3: Caseous necrosis only-42/592 and Group 4: Suppurative tuberculous lymphadenitis 39/592 cases. Acid fast bacilli (AFB) positivity on ZN staining was seen in 332 out of 592 (56.08%) cases.

Table- 2: Cytological spectrum of non-neoplastic lesions in lymph nodes.

Non-neoplastic Lesions	No. of Cases (%)
Tuberculous lymphadenitis	592 (40.06)
Reactive lymphadenitis	550(37.2)
Suppurative lymphadenitis	62 (4.2)
Cryptococcal lymphadenitis	1 (0.07)
Rosai-Dorfman's disease	1 (0.07)
Total	1206 (81.60%)

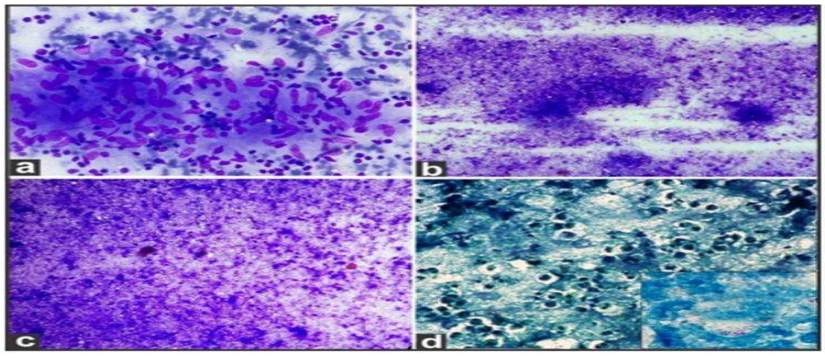

Fig-1: Tuberculous lymphadenitis patterns a) Epithelioid granuloma, Giemsa x 400 b) Epithelioid granulomas with caseous necrosis, Giemsa x 200 c) Caseous necrosis, Giemsa x 400 d) Suppuration with caseous necrosis, Pap x 400 (Inset: AFB positivity, ZN x 1000).

The next common lesion was reactive lymphadenopathy, seen in 550 (37.2%) cases mostly in the first decade of life. The other less common lesions included suppurative lymphadenitis 62(4.2%), cryptococcal lymphadenitis 1(0.07%) and Rosai-Dorfman's disease 1 (0.07%). One case of cryptococcal lymphadenitis was diagnosed in a 15 years old male patient who was a known case of HIV infection, presented with left inguinal lymph node swelling, confirmed by culture growth on chocolate agar and India ink stain. Another case was diagnosed as Rosai-Dorfman's disease in a three years old male child with right cervical lymph node swelling, but the follow-up of the case was not possible (Figure 2).

In neoplastic lesions, metastasis from other tumor sites was common with 243 (16.4%) cases, whereas, the primary neoplastic lesions of lymph node (malignant lymphoma) constituted only about 29 (2.0%) cases (Table 3).

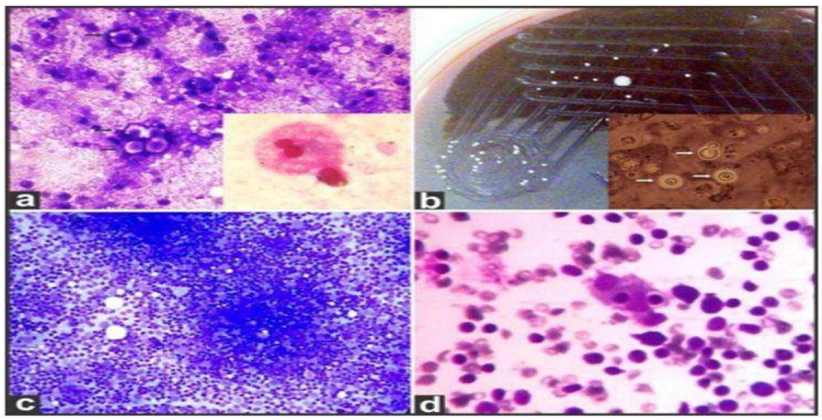

Fig-2: Cryptococcal lymphadenitis: a) Encapsulated budding yeasts (arrows), Giemsax400 (Inset: PAS positive yeasts within macrophage, PAS x 400) b) Mucoid colonies of *Cryptococcus neoformans* on chocolate agar (Inset: Clear halo around the yeasts (arrows), India Inkx400) c) Reactive lymphadenopathy, Giemsax100 d) Rosai-Dorfman's Disease, Giemsax400.

Table-3: Cytological spectrum of neoplastic lesions in lymph nodes.

Neoplastic Lesions	No. of Cases (%)
Primary malignant lesions	**29 (2.0)**
Hodgkins lymphoma	6 (0.40)
Non-Hodgkins lymphoma	23(1.60)
Metastatic lesions	**243 (16.4)**
Squamous cell carcinoma	184 (12.45)
Adenocarcinoma	44 (3.0)
Other lesions	15(0.98)
Total	**272 (18.40)**

The metastases were most commonly of squamous cell carcinoma in 184 (12.45%) cases, followed by adenocarcinoma in 44 (1.35%) cases. Other less common metastases were: poorly differentiated carcinoma 8 (0.54%), malignant melanoma 2 (0.13%), papillary carcinoma of thyroid 2 (0.13%), anaplastic carcinoma of thyroid 1 (0.06%), mucinous carcinoma of breast 1 (0.06%) and acute myeloid leukemia with spillage into lymph node 1 (0.06%). (Figure 3)

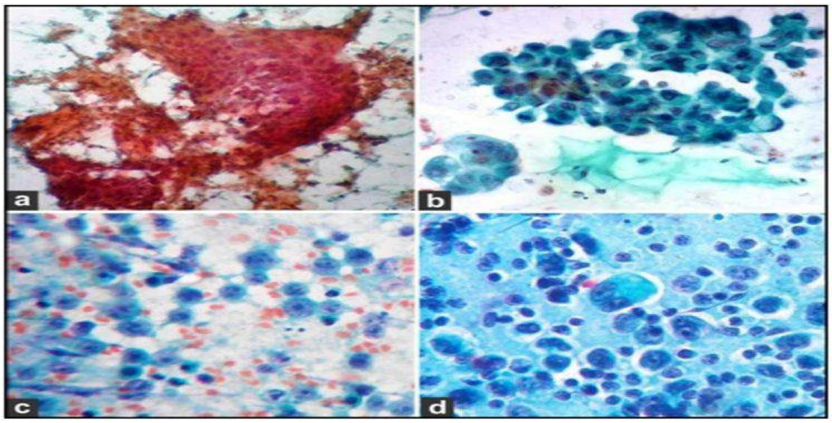

Fig-3: Malignant lesions a) Metastatic squamous cell carcinoma, Pap x 400 b) Metastatic adenocarcinoma with extracellular and intracellular mucin, Pap x 400 c) Non Hodgkin's lymphoma, Pap x 400 d) Hodgkin's lymphoma showing typical Reed-Sternberg cell, Pap x 400.

Out of total 29 cases of malignant lymphoma, 23 (1.60%) were Non-Hodgkin's lymphoma (NHL) and 6 (0.4%) were Hodgkin's lymphoma (HL) (Table 3). In one case of NHL, complete workup with biopsy and immunohistochemical (IHC) study was done. IHC showed CD20/ MUM1/ bcl-2 positivity, Ki-67 proliferative index upto 90% and focal c-myc positivity, and diagnosis of diffuse large B-cell lymphoma- activated B-cell (high-grade) type was made.

The tuberculous lymphadenitis showed highest incidence in the fourth decade, while reactive lymphadenitis was common in the first decade and metastatic lesions showed increase in the number of cases from fourth decade of life with highest number of cases (70/243 cases) seen in sixth decade. The malignant lymphomas were distributed evenly in all age groups from second decade. These findings had statistical significance with $P< 0.001$.

Discussion

FNAC is an important diagnostic tool to aid in the diagnosis of lymph node lesions. It is inexpensive, safe, quick and reduces the need for surgical biopsy [4]. Aspiration of lymph nodes was first done by Grieg and Gray in 1904, in a patient with sleeping sickness [2]. It was Dudgeon and Patrick in 1927, who first used FNAC in diagnosing tuberculous lymphadenitis [5]. In the present study, we made an attempt to present our experience with 1478 FNAC cases of palpable lymph node swellings, whose diagnosis was based on cytomorphological findings and highlighted the burden of disease in our institute.

In this study, maximum number of cases were recorded in age group of 21-30 years which was similar in studies done by others like Pavithra et al[6], Dukare et al, [7] Pandav et al, [8] Rajshekaran et al[9] and Chandanwale et al[10]. The overall lesions were slightly common in males compared to females, which was similar to that observed by Patel et al[11], Pandav et al[8], Giri et al[12], Hirachand et al [1] and Tilak et al[13]. While, the study done by Sharma et al [4] and Pavithra et al [6] showed that females were commonly affected with male: female ratio of 0.87:1 and 1:1.18 respectively. The male predominance could be accounted for more susceptibility of males due to high outdoor activities compared to females.

Cervical lymph nodes were the most common group of lymph nodes involved (69.0%) followed by axillary region (7.6%). Similar findings were also observed by Pavithra et al (85.27%)[6] Chandanwale et al(96.72%), [10] Kocchar et al (80.22%)[14] and Mohanty et al (66.48%) [15].

Tuberculous lymphadenitis was the most common lesion reported in 592(40.06%) cases and maximum numbers of these cases (190) were in age group 31-40 years with female preponderance (Male: female ratio of 1:1.1), followed by 21-30 years (174 cases) and 11-20 years (77 cases). Female preponderance of tuberculous lymphadenitis has been observed by Pavithra et al[6]. Fatima et al[16] and Chand et al[17]. This may be due to malnutrition and overall low living standards among females in this area.

In India, tuberculous lymphadenitis is one of the most common causes of lymphadenopathy encountered in clinical practice, with sharp contrast to its very low frequency of 1.6% in developed countries[18]. Most often it involves the cervical group of lymph nodes attributed to the rich lymphatic supply of the region [19].

Cytological pattern of tuberculous lymphadenitis was studied in detail in our study. The diagnosis of tuberculous lymphadenitis was made in the presence of caseous necrosis, epithelioid granulomas, caseous necrosis with epithelioid granulomas and suppuration (necrotic material in the background with degenerating and viable polymorphs) with AFB positivity on ZN stained smears[20].The predominant cytological pattern in our study was epithelioid granulomas with caseous necrosis seen in 270 (45.61%) cases.

ZN staining for AFB was seen in 56.08% cases in our study, while Chand et al reported 44.54% cases [17]. Bezabih et al reported 59.5% cases, [21] Paliwal et al reported 71% cases[22]. On the other hand, a very low AFB positivity rate of 19.6% was reported by Aggarwal et al in their study[23]. In our study, caseous necrosis alone was seen in 42 cases and all of these were positive for AFB.

It has been observed that the foci of caseous necrosis were associated with marked proliferation of tubercle bacilli whereas lymphocytes, epithelioid cells and multinucleated giant cells have a role in limiting the

proliferation of AFB [24,25]. Therefore, it is expected that FNAC from tuberculous abscess yields more AFBs than early tuberculous lymph nodes [26]. Granulomas can be seen in variety of other conditions causing lymphadenopathy including sarcoidosis, carcinoma, lymphoma, fungal diseases, cat scratch disease, collagen vascular disease and disease of the reticuloendothelial system. But, in the region where tubercular infection is common and other granulomatous diseases are rare, the presence of a granulomatous feature in FNAC is highly suggestive of tuberculosis[2].

In our study, a total of 241 cases (40.71%) had presented with epithelioid granulomas alone, but amongst them 19 cases (7.9%) of them were AFB positive. In epithelioid granulomas without necrosis, AFB positivity was very low[20] and in cases negative for AFB other granulomatous diseases were ruled out based on clinical history, chest X-ray and a diagnosis of tuberculosis was considered.

Reactive lymphadenopathy was seen in 550 (37.2%) cases which was comparable to study by Vimal et al (33.64%) [20] and Giri et al (34.59%)[12]. Maximum number of cases (177) were seen in the first decade of life in males. While in other studies, reactive lymphadenopathy was most frequent diagnosis and its incidence ranged from 18.9% to 42% [1,14,15,27].

Acute suppurative lymphadenopathy was observed in 62 (4.2%) cases in our study which was comparable with the study done by Patra et al (5.8%)[28] and Kocchar et al (4%)[14]. Metastatic malignancies were the third most common lesions affecting lymph nodes, seen in 243 cases (16.4%) with highest number of cases in age group 51-70 years (137 cases).This was comparable to study done by Giri et al (21.8%) [12] and Vimal et al (17.65%)[20].

Predominant metastatic deposits were of squamous cell carcinoma (75.7%) of all metastatic lymph nodes, which correlates with findings of studies by Pavithra et al (56.25%)[6]. The high incidence of squamous cell carcinoma may be due to high incidence of smoking and tobacco chewing in this region. The primary malignant lesions (malignant lymphoma) constituted 29 cases (2%) in our study, which was comparable to study conducted by Khajuria et al (2%) [27] Giri et al (2.7%)[12] and Sharma et al (2.7%).[4]

In the present study, NHL and HL constituted 1.6% (23 cases) and 0.4% (6 cases) respectively. This was comparable to study by Sharma et al, in which, NHL and HL constituted 2.3% and 0.4% respectively. [4] However, Hafez et al reported that cases suspicious for NHL were 32.5% and were the prominent cause of cervical lymphadenopathy [29]. Limitation of our study is that only few cases of lymphoma were excised for immunohistochemical typing.

To conclude our study, the most common cause for enlarged lymph nodes were tuberculous lymphadenitis and reactive lymphadenopathy followed by malignant neoplasm, especially metastatic squamous cell carcinoma. Our study showcased the burden of tuberculosis in our region and highlighted the usefulness of FNAC in reaching prompt diagnosis in lymphadenopathy patients. FNA of lymph node provides material for Catridge based nucleic acid amplification test (CBNAAT) in cases of multidrug resistant tuberculosis (MDRT) which is thegrowing threat in health care and in prompt management of MDRT cases. In cases of primary malignant lesion and metastases, lymph node aspiration also provides material for special studies such as cytochemistry, immunopathology or culture and assist in the guidance of therapy in time, thus reducing mortality and morbidity. From the results of this study and those from the literature, FNAC is recommended as the initial reliable diagnostic tool in peripheral lymphadenopathy.

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