

Original research article

## Evaluation of Cytological Spectrum of Lymph Node Lesions and Correlation with CD4+ T Cell Count in HIV Positive Patients.

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### Abstract

**Objective:** To evaluate the cytomorphological spectrum of HIV-related Lymphadenopathy and to correlate the FNAC findings with CD4+ T cell count.

**Material and methods:** The cross-sectional study was conducted on 69 HIV positive patients presenting with superficial lymphadenopathy and lymph nodes were sampled in each case for fine needle aspiration cytology (FNAC). All the smears were stained with May-Grunwald-Giemsa (MGG) & Papanicolaou (Pap) stain and then evaluated under light microscopy; and a cytological diagnosis was rendered. Ziehl-Neelsen (ZN) staining and Mycobacterial culture was also done in 59 and 42 cases respectively. CD4+ T cell (CD4+) count was also evaluated in 59 cases.

**Result:** The aspirates were adequate in 63(91.3%) patients. Caseating granulomatous lymphadenitis was the most common cytological diagnosis found in 28 cases (40.58%), followed by reactive lymphoid hyperplasia in 16 cases (23.19%). Mean CD4+ count of caseating granulomatous lymphadenitis was 162.9 cells/ $\mu$ L and mean CD4+ count of non Hodgkin lymphoma NHL cases was 239 cells/ $\mu$ L. There was one case of histoplasmosis in our study where CD4+ count was found to be 86 cells/ $\mu$ L. A significant association was found between these FNAC findings and their respective CD4+ count distribution upon Fisher's Exact Test (P value = 0.044).

**Conclusion:** FNAC is an important diagnostic modality for assessing the cause of lymphadenopathy in HIV. A low CD4+ count is indicative of immunosuppression in HIV patients and may predict the occurrence of opportunistic infections. TB can be suspected when CD4+ count is around 200 cells/ $\mu$ l.

**Keywords:** HIV, lymphadenitis, FNAC, CD4+ Count, granulomatous.

## Introduction

About 37.7 million people globally were living with HIV in 2020 [1]. In 1986 first AIDS case in India was detected since then, HIV infection has been reported in all states and union territories. The total number of people living with HIV in India was estimated at 23.19 lakh (18.33 lakh– 29.78 lakh) in 2020. Children (<15 years) accounted for 3.5% of total infection and 44.3% of total infections were among females[2]. Acquired immunodeficiency syndrome AIDS is known to be caused by a lymphotropic retrovirus known as human immunodeficiency virus HIV and characterized by profound immunosuppression that leads to opportunistic infections, secondary neoplasm and neurological manifestation [3]. HIV infects cells by using the CD4 molecule as receptor and chemokine receptors CCR5, CXCR4 as coreceptor. CD4+ count helps in predicting immunological damage during infection and monitoring recovery after antiretroviral treatment [3,4].

One of the important manifestations of HIV infection is lymphadenopathy [5]. Fine-needle aspiration cytology (FNAC) is a simple and cost effective investigation for HIV associated lymphadenopathy [6]. The present study describes the cytopathological patterns associated with HIV lymphadenopathy and also demonstrates its correlation with CD4+ count. Lymphnode cytology is useful for segregating HIV lymphadenopathy cases that needs further evaluation. The aim of the present study was to evaluate the role of fine needle aspiration cytology in diagnosis of lymphadenopathy in HIV infected patients and to find out the correlation between cytological findings and CD4+ count.

## Material and methods

This cross-sectional study was conducted on HIV positive patients presenting with superficial lymphadenopathy, attending the outpatient department of medicine of SS Hospital, IMS, BHU and referred to department of pathology for FNAC from January 2010 to July 2011. A well informed consent was taken from each of the participating individuals. In all these patients a detailed history was taken. All participants were clinically examined and thereafter investigations were carried out including FNAC and CD4+ count. FNAC was performed on superficial lymph nodes as routine outdoor procedure. It was done with 22 gauge needle attached with 10 ml disposable syringe. Universal precaution was followed during the procedure and while performing staining. Three aspirates per patient were collected. From the first aspirate six smears were prepared. Out of those six smears, two were air dried for MGG staining, two were fixed in alcohol for Pap staining, one was air-dried for ZN staining and the last one was kept unstained for PAS staining (if required). Second aspirate was inoculated in Lowenstein-Jensen (L-J) media for mycobacterial culture. Third aspirate was inoculated in McCartney bottle containing brain heart infusion (BHI) broth for bacterial culture other than mycobacteria.

**Microscopic analysis:** All the smears were evaluated using light microscopy and a cytological diagnosis was rendered for each case.

## Results

There were 69 patients with age ranged from 8 months to 62 years. The maximum number of patients [33 (47.83%)] were aged between 31 to 40 years. The mean and median age of presentation was 35.29 and 35 yrs respectively. Two patients belonged to paediatric age group (<15 years), out of which one was infant. Out of 69 patients included in study 48 (69.57%) were male and 21 (30.43%) were female.

Out of 59 patients who were enquired for the history of intake of HAART, 31 patients were on HAART drugs. Most common involved site was cervical lymph node, which was found to be

involved in 34 (49.27%) patients. Axillary lymphadenopathy was found in 18 (26.09%) patients, where as involvement of inguinal lymph nodes was seen in only 3 (4.35%) patients. Axillary lymphadenopathy along with cervical lymph node involvement were found in 8 (11.59%) patients, while axillary as well as inguinal lymphadenopathies were found in only 2 (2.90%) patients. Cervical as well as inguinal lymphadenopathies were found in 3 (4.35%) patients. One (1.45%) patient had generalized lymphadenopathy.

Aspirates from 6 (8.6%) patients were scanty and inadequate for the diagnosis. Aspirates of 63 patients (91.3%) were adequate. Caseating granulomatous lymphadenitis appeared to be most common microscopic finding, occurring in 28 (40.57%) patients (Table 1). Reactive lymphoid hyperplasia was found in 16 (23.19%) cases. Two types of necrotizing lymphadenitis have been identified; one was caseating necrotizing lymphadenitis type and another type was suppurative necrotizing lymphadenitis, both of these subtypes were represented by 5 cases each. Suppurative granulomatous lymphadenitis was found in 4 (5.80%) cases. Granulomatous lymphadenitis without necrosis was observed in one case.

Cytological impression of non Hodgkin lymphoma was made in 2 (2.90%) cases. Metastatic squamous cell carcinoma was detected in one male patient who underwent penectomy for squamous cell carcinoma of penis.

In one case lymph node aspirate showed scattered macrophages containing multiple intracellular organisms. The cytoplasm of these macrophages was abundant. The intracytoplasmic organisms were round to oval 2-4  $\mu$ m in diameters and identified as histoplasmic yeasts.

**Table 1: Cytomorphological patterns of lymphadenopathy in HIV patients.**

FNA diagnosis	Total (n=69)	Percentage
Caseating granulomatous <a href="#">lymphadenitis</a>	28	40.57
Suppurative Granulomatous Lymph adenitis	4	5.80
Necrotising Lymphadenitis (caseating)	5	7.24
Necrotising Lymphadenitis (suppurative)	5	7.24
Granulomatous lymphadenitis without necrosis	1	1.45
Reactive	16	23.19
NHL	2	2.90
Fungal (Histoplasmosis)	1	1.45
Others (metastatic SCC)	1	1.45
Inadequate	6	8.69

**Table 2: Correlation of Fine needle aspiration cytology diagnosis with Zeihl-Neelsen (ZN) stain findings.**

FNA diagnosis	Total	Zn stain (+ve) for AFB		Zn stain (-ve) for AFB		Not done
		No.	%	No.	%	
Caseating granulomatous lymphadenitis	28	24	85.71	4	14.29	-
Suppurative Granulomatous lymphadenitis	4	3	75	1	25	-
Necrotising lymphadenitis (caseating)	5	4	80	1	20	-
Necrotising lymphadenitis (suppurative)	5	3	60	2	40	1
Granulomatous lymphadenitis without necrosis	1			1	100	-
RLH	16			16	100	-

RLH- Reactive lymphoid hyperplasia; NHL- Non-Hodgkin Lymphoma

Zeihl-Neelsen staining on aspiration smears and their relation to cytological diagnosis was observed in different groups (Table 2).

Samples from 42 cases were inoculated in L-J media for mycobacterial culture of which 14 were positive for mycobacterial colonies

Out of 42 cases, 35 cases showed either ZN positivity or culture positivity or both. 7 cases were neither ZN positive nor culture positive. Thirty four cases were positive for AFB on ZN staining and 14 cases were culture positive. Twenty one cases were only ZN positive and 1 case was only culture positive. Thirteen cases were positive for AFB on ZN staining as well as culture in L-J media. One case showing negativity for ZN stain was positive for mycobacterial culture.

43 samples were inoculated in BHI broth for bacteriological culture. In 4 samples colony growth was found to occur, 2 were contaminated & rest were sterile. In one case of caseating granulomatous lymphadenitis colonies of staphylococcus aureus (staph aureus) were found and in another one with same diagnosis, colonies of Esterechia coli appeared. Colonies of streptococcus faecalis were found in one case of suppurative necrotizing lymphadenitis, while those of staph. aureus were detected in the case of granulomatous lymphadenitis.

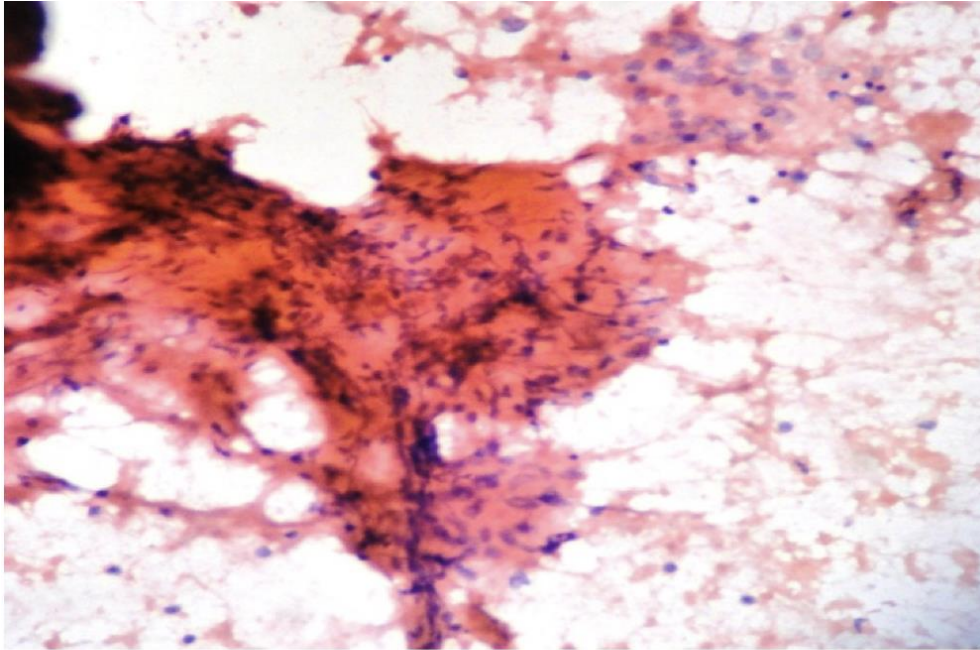
**Table 3. Correlation of CD4+ counts with fine needle aspiration cytology findings.**

FNAC Category	Number (n = 59)	CD4+ count (cells / $\mu$ l)				Mean
		<100	101-200	201-500	>500	
Caseating granulomatous lymphadenitis	22	7	8	7	-	162.9
Granulomatous lymphadenitis	1	-	-	1	-	221
Caseating necrotizing lymphadenitis	5	3	1	1	-	128.40
Suppurative necrotizing lymphadenitis	5	2	2	1	-	177.66
Suppurative granulomatous lymphadenitis	3	1	1	1	-	130.40
Reactive lymphoid hyperplasia	14	-	4	5	5	386
Non-Hodgkin's-lymphoma	2	-	-	2	-	239
Metastatic Squamous cell carcinoma	1	-	-	1	-	490
Histoplasmosis	1	1	-	-	-	86
Inadequate	5	2	1	1	1	
Total	59	16	17	20	6	

Out of 69 subjects, CD4+ counts of only 59 cases could be ascertained and that of 10 cases were not available. CD4+ counts ranged from a minimum of 12 cells/ $\mu$ l to a maximum of 800 cells/ $\mu$ l (Table 3). The mean and median CD4+ counts were 219 and 180 cells/ $\mu$ l respectively. Patients with reactive lymphoid hyperplasia had highest mean CD4+ counts of 386 cells/ $\mu$ l. However there was only one case of metastatic squamous cell carcinoma which had a CD4+ count of 490 cells/ $\mu$ l. Mean CD4+ count of NHL was 239 cells/ $\mu$ l. Lowest CD4+ count of 86 cells/ $\mu$ l was found in fungal case of Histoplasmosis. Among cytological patterns of tubercular lymphadenitis, patients with caseating necrotizing lymphadenitis demonstrated lowest mean

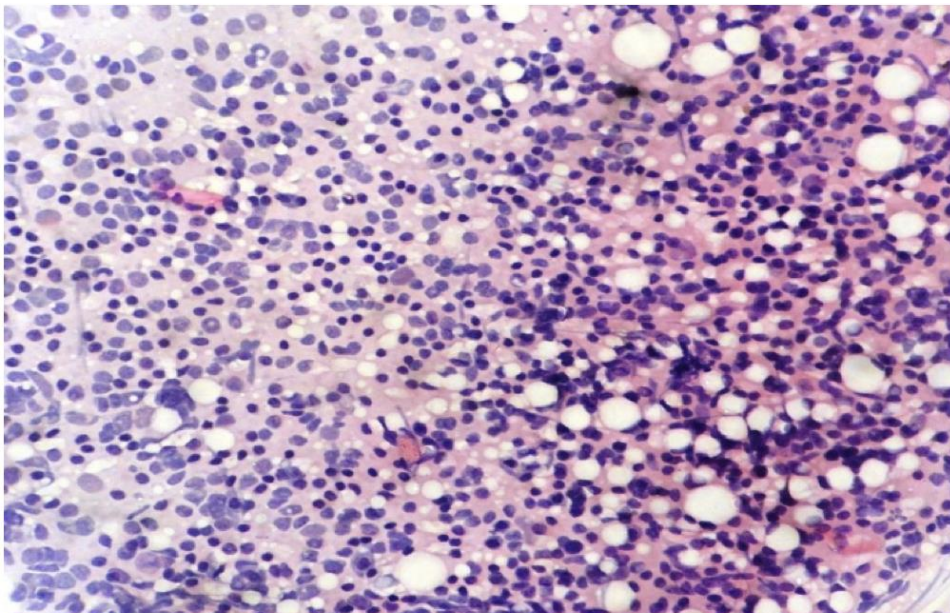
and median CD4+ count of 128.40 cells/ $\mu$ l and 85 cells/ $\mu$ l respectively; followed by cases of suppurative granulomatous lymphadenitis with mean CD4+ count of 130.40 cells/ $\mu$ l. In cases categorised as caseating granulomatous lymphadenitis a mean CD4+ count of 162.9 cells/ $\mu$ l was observed, whereas patients with a cytological diagnosis of suppurative necrotizing lymphadenitis had a mean CD4+ count of 177.66 cells/ $\mu$ l.(Table 3)

A significant association was found between these FNAC findings and their respective CD4+ count distribution upon Fisher's Exact Test (P value = 0.044).



**CASEATING GRANULOMATOUS LYMPHADENITIS**

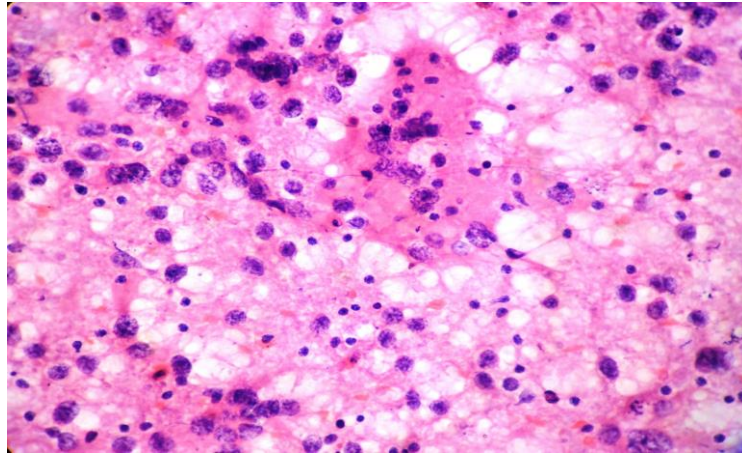
Microphotograph showing epithelioid cell granulomas. Caseous necrosis noted in the background ( 400x, Pap Stain)



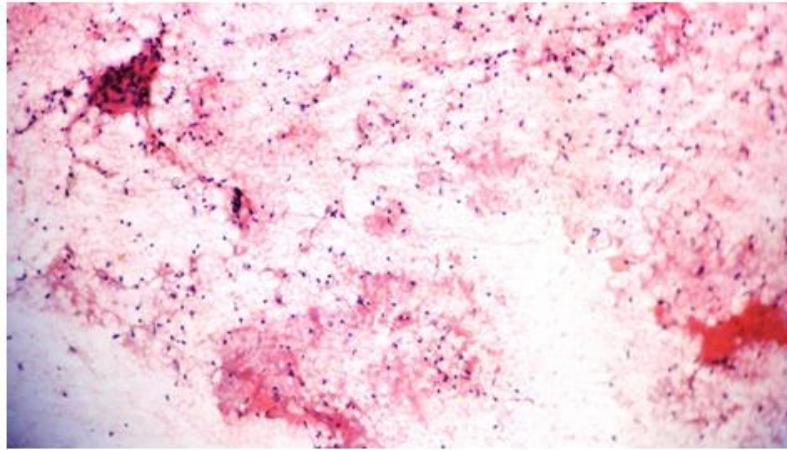
**REACTIVE LYMPHOID HYPERPLASIA**

Photomicrograph showing polymorphous population of lymphoid cells including occasional histiocytes and plasma cells(High Power, Pap Stain)

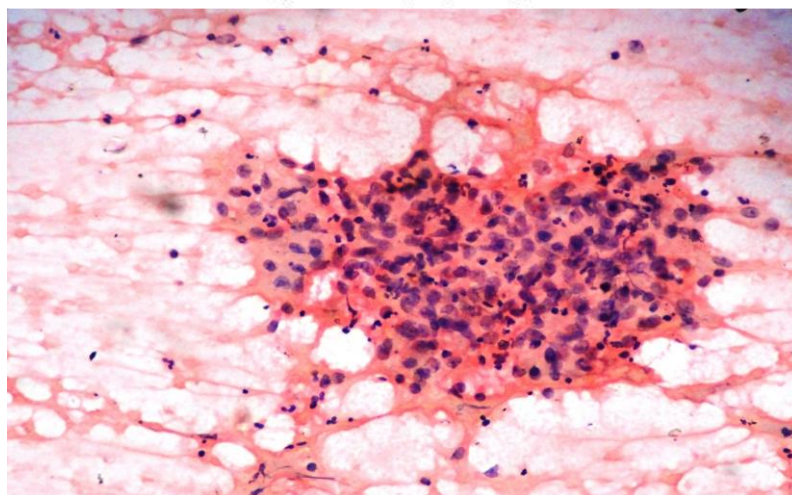




**NON-HODGKIN'S LYMPHOMA:**  
Photomicrograph from case no. showing dispersed population of atypical lymphoid cells displaying high N:C ratio, irregular clumped chromatin admixed with a few small lymphocytes.  
(High Power, Pap Stain)



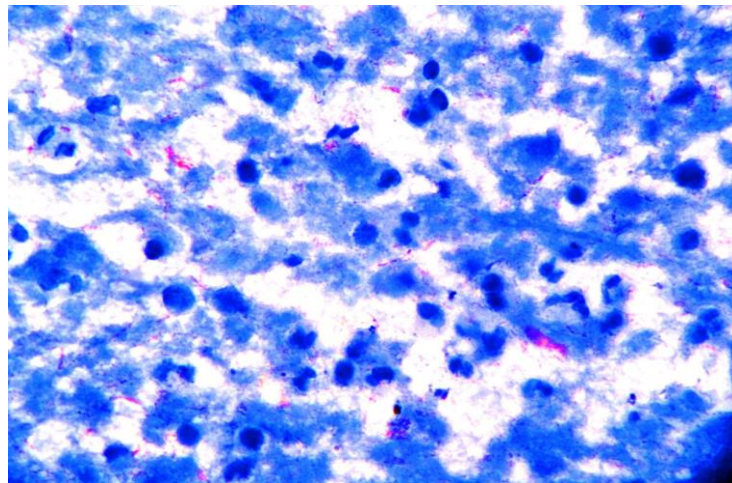
**Microphotograph showing epithelioid cell granulomas present over a caseous necrotic background**  
(Low Power, Pap Stain)



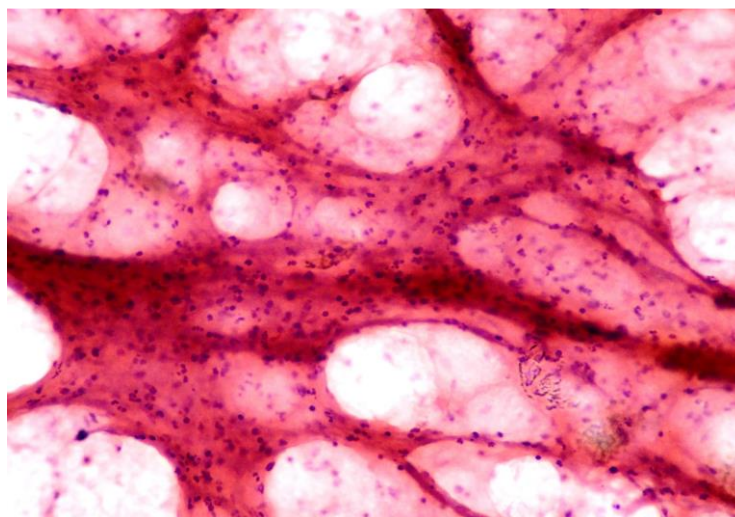
**SUPPURATIVE GRANULOMATOUS LYMPHADENITIS:**  
Photomicrograph showing an epithelioid histiocytes granuloma admixed with it are present few neutrophils on a necrotic background.  
(High Power, Pap Stain)



L J media showing multiple nodular creamish white mycobacterial colonies

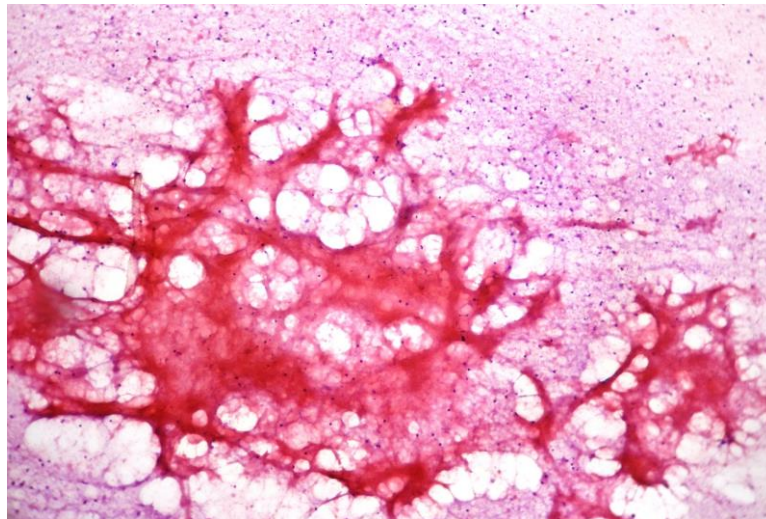


**TUBERCULAR LYMPHADENITIS:**  
Photomicrograph showing many singly scattered and clumps of Acid fast bacilli  
(1000 X Zeihl-Neelsen Stain )

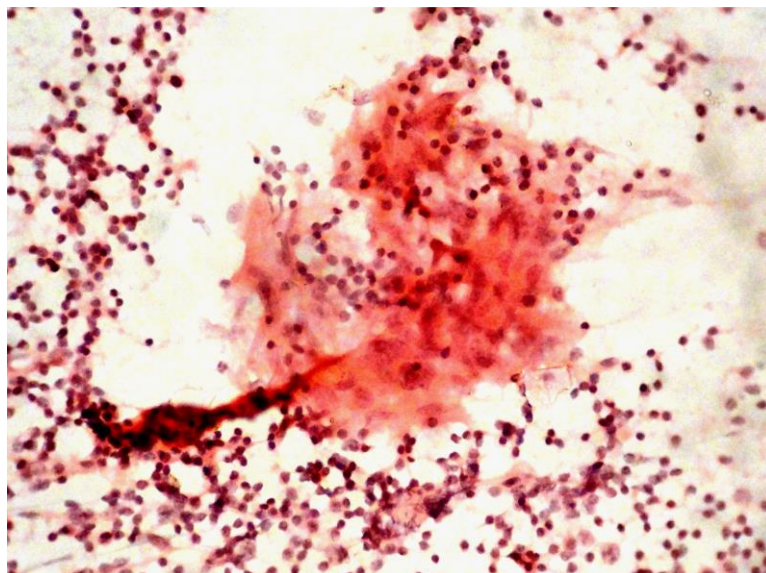


**SUPPURATIVE NECTROTIZING LYMPHADENITIS:**  
Photomicrograph showing predominantly necrotic debris admixed with dense infiltrate of  
neutrophils with occasional histiocytes.  
(High Power, Pap Stain)

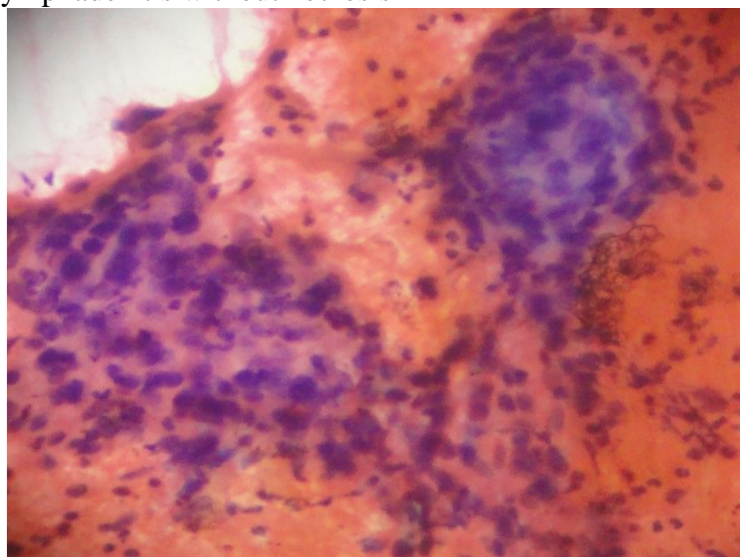




**CASEATING NECROTISING LYMPHADENITIS:**  
Fig. 4 : Microphotograph showing caseous necrosis. No granuloma or viable cell seen (200X , Pap Stain)

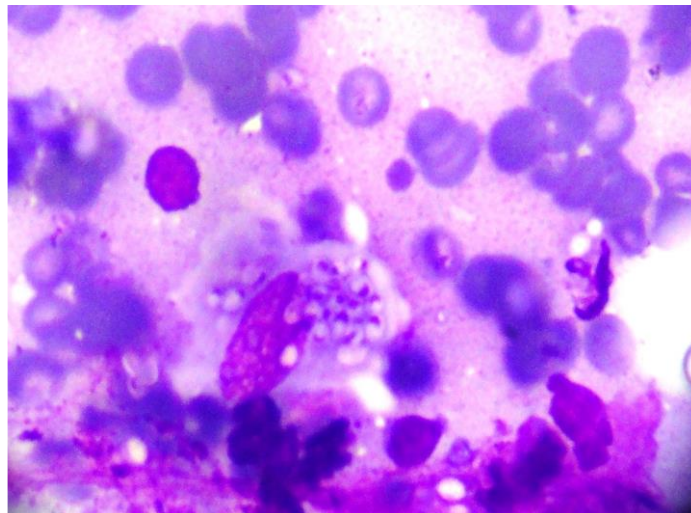


Granulomatous Lymphadenitis without necrosis





## Metastatic Squamous Cell Carcinoma



Microphotograph of Histoplasma (Multiple histoplasma yeasts in a macrophages)

**Discussion:**

FNAC is an important, reliable, rapid, and inexpensive method of establishing the diagnosis of lesions at various sites [7,8]. Out of HIV positive group, age group of 31-40 yrs. was most commonly involved (47.83%). The mean age of our participants was 35.29 yrs. This study was similar to Neelima et al [9] (76% between 21-40 yrs) and similar findings were reported by Deshmukh et al [10]. Out of 69 patients 48 (69.57%) were male and 21 (30.43%) were female.

In our study 49.28% patients showed cervical lymphadenopathy alone followed by axillary lymphadenopathy which was observed in 26.09% patients. Cervical with axillary lymphadenopathy was observed in 11.59% cases whereas bilateral inguinal lymphadenopathy was seen in 4.35% patients. Cervical with inguinal lymphadenopathy was also noted in 4.35% of our cases. These findings of present study go well in concurrence with similar study done by Patil et al [11] that reported cervical lymphadenopathy in 51.80% and axillary lymphadenopathy in 22.20% of their cases, while in 7.40% of their participants inguinal lymph nodes were involved. These observations regarding lymph nodes in our study are also well comparable with other studies like Kumarguru et al, Dutta et al, Parikh et al, Deshmukh et al, Baghel et al, Ratan et al and Gorva et al [5,12,13,10,14,15,16]. However our findings showed discordance with the study conducted by Satyanarayna et al [17] where they found multiple site involvement of lymph nodes (46.8%) followed by axillary (36.8%) and cervical (12.6%) lymph nodes.

Tuberculous lymphadenitis (50.72%) was the most common finding in the present study followed by reactive lymphoid hyperplasia (23.19%). These findings are well comparable with previous studies of Gorva et al [16] that reported tubercular lymphadenitis in 56% and reactive lymphoid hyperplasia in 28% of their cases. Other studies performed by Shenoy et al, Jayram et al and Vanisri et al. also showed similarity with our findings, where tubercular lymphadenitis was found to be most common observation [18,19,20]. However our findings are not in concurrence with those of Bottles et al, Shobhna et al, Satyanarayna et al and Sarma et al where reactive lymphadenopathy was the most common cytological finding [21,22,17,23].

Among tubercular lymphadenopathy cases, most commonly observed cytological pattern in our study was caseating granulomatous lymphadenitis (68.57%), which was quite comparable with those reported by Shenoy et al [18] (70.83%). Similarly other studies like Vanisri et al

[20] and Shobhna et al [22] have also observed caseating granulomatous lymphadenitis as most common cytological pattern among tubercular cases. However Satyanarayna et al [17] found caseating necrotizing lymphadenitis as the most common cytological pattern, where as Jayram et al [19] reported suppurative necrotizing lymphadenitis as the most common cytological pattern. All the sixteen cases of reactive lymphadenitis in our study showed negative result for acid fast bacilli on ZN staining. Satyanarayna et al [17] have observed a reactive cytological pattern in 16.4% of their cases of tuberculosis.

Apart from tuberculosis, one case of Histoplasmosis was also found in the present study as another opportunistic infection as seen in studies done by Jayram et al [19] and Sarma et al [23] where Histoplasma was reported in 2 and 1 case respectively. Both these studies also reported cryptococcal infection like various other studies.

HIV patients have greater risk of developing lymphoma. Lymphoma can be diagnosed on fine needle aspiration by its cytological features alone. In present study non-Hodgkin lymphoma (NHL) was observed in two cases (2.9%), which correlates well with the findings of Vanisri et al, Jayram et al, Sarma et al, Shobhna et al. Parikh et al, Saikia et al and various other studies [20,19,23,22,13,6]. In a study done by Patil et al [11] they reported a higher percentage (16.6%) of lymphoma out of which 5.5% cases were of Hodgkin lymphoma and 11.1% cases were categorised as NHL. Likewise Shenoy et al [18] reported 10% cases as NHL.

One (1.45%) case of metastatic carcinoma (metastatic squamous cell carcinoma) was found in our study, where as Patil et al, Gupta et al, Shenoy et al and Ratan et al reported 5.5%, 0.8%, 2% and 1.3% cases of metastatic caecinoma respectively in their studies [11,24,18,15].

We were keen to know whether the level of immunosuppression had any correlation with specific cytological findings. CD4+ count was good in patients with reactive lymphoid hyperplasia with mean count 386 cells/ $\mu$ l and patient with histoplasma infection had the lowest CD4+ count of 86 cells/ $\mu$ l. The plausible explanation for this could be that low CD4+ count indicates pronounced immunosuppression predisposing the HIV patients to opportunistic fungal infections like Histoplasma. This is in concordance with study done by Gorva et al. & Ratan et al [16,15]. Among cytological patterns of tubercular lymphadenitis, patients with caseating necrotizing lymphadenitis demonstrated lowest mean CD4+ count of 128.40 cells/ $\mu$ l; followed by cases of suppurative granulomatous lymphadenitis with mean CD4+ count of 130.40 cells/ $\mu$ l as seen in study done by Kumarguru et al [5].

## Conclusion

There was increased prevalence of tubercular lymphadenitis in HIV positive patients. Correlation of CD4 counts provides information about the immune status and stage of the disease. Thus FNAC is an effective diagnostic modality for HIV positive lymphadenopathy patients and helps in identifying majority of the reactive and neoplastic lesions and guides for the subsequent management of the patient. TB can be suspected when CD4+ count is around 200 cells/ $\mu$ l.

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