

## ORIGINAL ARTICLE

# Fine Needle Aspiration Cytology of Lymphnode in HIV Positive Patients and Its Correlation with CD4 Count

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## ABSTRACT

**Background:** AIDS is a fatal disease with many opportunistic infections. Causes of lymphadenopathy are opportunistic infections or lymphoid malignancy. FNAC is useful tool for the diagnosis of reactive lymphadenitis, acute suppurative lymphadenitis and opportunistic infections in HIV. There is a correlation between CD4 and FNAC findings. There is low CD4 count in reactive hyperplasia, suppurative lymphadenitis & granulomatous inflammation & very low count in lymphoma and Kaposi's sarcoma.

**Aims & Objectives:** To find out the pathological changes such as infections, benign and malignant lesions in the lymph nodes of HIV positive patients by FNAC and to establish a correlation between clinicopathological findings, FNAC & CD4 count.

**Methodology:** The study of 100 patients was carried out in the Department of pathology, SMIMER Medical College, Surat. FNAC of the lymph nodes were performed and smears were stained by Giemsa, H&E, PAP & ZN stains to rule out fungus & AFB. CD4 count of the same patients were performed.

**Results:** Out of 100 patients, maximum cases of 31 to 40 years (32%) with M:F of 2:1. cervical LNs (77%) with findings of Tuberculous lymphadenitis (70%), reactive hyperplasia (12%) & Lymphoma (5%) cases. Lymphoma were associated with severe reduction of CD4 count (66 to 145).

**Conclusion:** Incidence of infectious lesions was higher (76%) with maximum number of patients were of Tuberculous lymphadenitis with CD4 count range of 200 to 499 and cases of lymphoma were associated with CD4 count (66 to 145).

**Keywords:** AIDS, CD4, lymphnode, tuberculosis, lymphoma

## INTRODUCTION

AIDS was first recognized in the United States in the summer of 1981. In 1983, human immunodeficiency virus (HIV) was isolated from a patient with lymphadenopathy, Gia Carangi was a supermodel and one of the first famous women to die of HIV/AIDS<sup>1</sup>. In 1985, a sensitive enzyme-linked immunosorbent assay (ELISA) was developed, which led to an appreciation of the scope and evolution of the HIV epidemic at first in the United States and other developed nations and ultimately among developing nations throughout the world<sup>2</sup>.

AIDS is a fatal illness that breaks down body's immunity and leaves the victim vulnerable to life threatening opportunistic infections, neurological disorders or unusual malignancies.<sup>3</sup> In India the Human Immunodeficiency Virus (HIV) epidemic is now emerged as one of the most serious public health problems in our country.<sup>4</sup> Lymphadenopathy

may also be a manifestation of opportunistic infections, lymphoid malignancy developing in a immunocompromised individual. Fine needle aspiration cytology (FNAC) can serve as an alternative method and may be practiced for the diagnosis of reactive lymphadenitis, acute suppurative lymphadenitis and opportunistic infections in HIV/ AIDS viz. tuberculosis, histoplasmosis, toxoplasmosis and malignant conditions such as kaposi sarcoma, and lymphoma.<sup>5</sup>

There is a correlation between CD4 count and FNAC findings. It is seen that there is a general downward trend in mean CD4 count from reactive hyperplasia or suppurative lymphadenitis to granulomatous inflammation and further very low count in lymphoma and Kaposi's sarcoma.

The present study was designed to find out the pathological changes such as infections, benign and malignant lesions in the lymph nodes of HIV positive patients by FNAC; to establish a correlation be-

tween clinico-pathological findings and FNAC findings; and to establish a correlation of fine needle aspiration cytological findings with the CD4 count.

**METHODOLOGY**

**Sample size:** This is prospective cross-sectional observational study comprises a total of 100 patients. This study was carried out in the Department of Pathology, SMIMER Medical College, Surat during the period June 2012 to October 2013. All the patients enrolled in study were evaluated and few of them were followed in a prospective manner over a period of 17 months.

**Patient’s source:** The cases directly coming to an out patient department of medical or surgical departments as well as indoor patients referred from the other departments with cervical and axillary lymphadenopathy who were vitally stable.

Patient reactive for ELISA test of HIV, patients with cervical and axillary Lymphadenopathy of > 1 cm size and vitally stable patients were included in the study.

**Methods:** FNAC of the lymph nodes were performed and 5-6 smears were immediately kept in fixative (methanol), at least 2 wet smears were stain for H & E stain and 2 air dried smear for Giemsa stain and rest smears were kept unfixed for any special stain like PAP Stain, Zeil-Neelsen(ZN) stain for AFB and PAS stain for fungi if required. The smears were kept in methanol for 15 min for fixation. Then they were allowed to dry and staining was carried out.

**Calculation of CD4 count:** All the 100 seropositive patients included in this study were then sent to Antiretroviral Therapy (ART) centre in SMIMER Hospital, Surat for CD4 cell count testing. They collect

the blood & send the Blood to the ART center of Civil Hospital, Surat for the CD4 count. The procedure done for CD4 count is FACS (fluorescence activated cell sorter count system) caliber method.

**Collection & transport of blood for CD4 count:**

Label the collection tube with the date, time of collection, and a unique patient identifier/name, age & sex. The whole blood collected in EDTA was sent to the laboratory within six hours. The test was done within 48 hours of collection of blood.

**RESULTS**

The prospective cross sectional observational study of fine needle aspiration cytology of lymphadenopathy conducted between 2012 to 2013 at Department of Pathology, SMIMER Medical College, Surat.

The Range of age of patients was from 4 month-70 years with median age of 35 .The incidence of lymphadenopathy decreased with advancing age after 50 years. The male to female ratio was approx 2:1.

**Table 1: Age distribution**

Age(years)	No.
0-10	8
11-20	10
21-30	24
31-40	32
41-50	17
51-60	7
61-70	2
<b>Total</b>	<b>100</b>

**Table 2: FNA Cytology Of Lymphnode Finding In HIV Patients (n=100 cases)**

Lesion	No.	Axillary	Cervical
Acute suppurative lymphadenitis	6	2	4
Benign lympho-epithelial lesion	2	0	2
Reactive hyperplasia	12	2	10
Tuberculous lymphadenitis	70	14	56
Lymphoma	5	0	5
Cystic swelling	1	0	1
Keratinous cyst	1	0	1
Metastatic deposit	3	0	3

**Table 3. Lymphoma Diagnosis**

	CD4 count	No. of Lymphnode	Splenomegaly
Anaplastic large cell lymphoma	145	Single	Y
Large cell lymphoma	104	Multiple	N
Non Hodgkins lymphoma	78	Single	Y
Hodgkins lymphoma	66	Multiple	Y
Diffuse large cell lymphoma	98	Multiple	N

**Table 4. Correlation Of CD4 Range & Cytological Diagnosis By FNAC Of Lymphnode**

CD4 range	Median CD4 count	Cytological diagnosis	Number diagnosis
116-520	338	Acute suppurative lymphadenitis	6
121-1162	641.5	Benign lympho-epithelial lesion	2
119-670	313	Reactive hyperplasia	12
49-640	210	Tuberculous lymphadenitis	70
66-145	98	Lymphoma	5
336	336	Cystic swelling	1
223	223	Keratinous cyst	1
78-360	105	Metastatic deposit	3

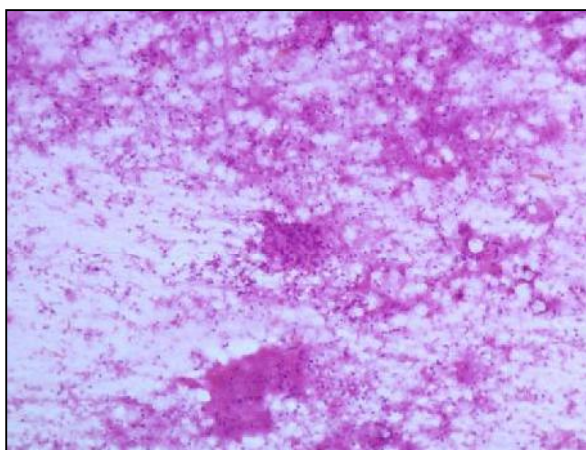


Fig 1: Tuberculous Lymphadenitis (H&E, 10X)

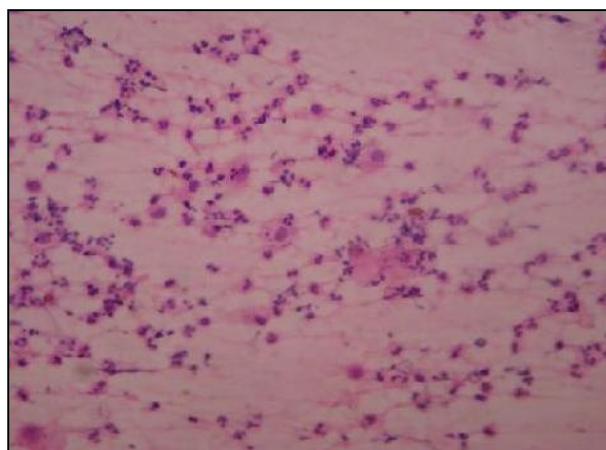


Fig 3: Suppurative lymphadenitis. (H&E, 40X)

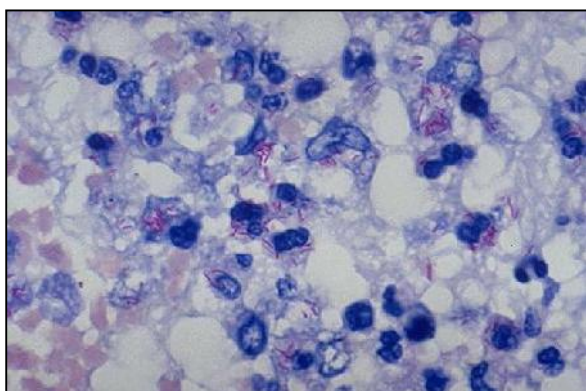


Fig 2: Numerous AFB on ZN stain. (100x)

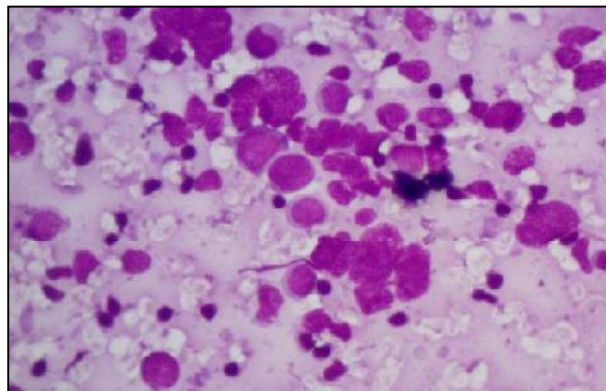


Fig 4: Cytological features of large cell lymphoma. (H&E 40X)

**DISCUSSION**

The fine needle aspiration cytology is a recent, world wide accepted, non hazardous, practically non invasive, inexpensive and diagnostically fairly accurate method. It has been proved that the reliability and accuracy of FNAC is very high in experienced hands. Out of 100 aspirates, cytological diagnosis of infectious, benign and malignant was given in 76%, 16% and 8% respectively. The most common cytological diagnosis was infectious 76% and tuberculosis was the most common etiology found among infectious disease.

The age range of the patient varied from 4 months to 70 years. Majority of the patients were in the age group 31-40 years (32%) followed by 21-30 years (24%). Similar findings were recorded by Amit et al<sup>9</sup>.

Similar findings were recorded by Guru et al. on 231 cases of lymphadenopathy in HIV positive patient. Majority of patients were of age group 21-40 years. In similar study by Vanisri et al.<sup>7</sup> on 36 cases of lymphadenopathy in HIV positive patients 44.4% cases were recorded in age group of 21-30 years closely followed by 31-40 years (25%). In the present study, males (66%) were affected more commonly than female (34%), male: female ratio was 2.16:1. Similar findings were reported by Amit et al. in which males (59.2%) were affected more commonly than female (31.66%), male: female ratio was 2.16:1, Guru et al.<sup>6</sup> in which it was 2.3:1, Parikh et al.<sup>8</sup> it was 3.44:1. In the present study the maximum number of patient presented with cervical lymph node enlargement 77% which correlates with Amit et al. 280 (93.4%),

Guru et al (78.76%), Parikh et al (62.5%), and many others like Vanisri et al, and Bates et al.

In the present study 76(76%) cases were diagnosed cytologically as infectious, non-neoplastic 16(16%) and 8(8%) as neoplastic, which was comparable with Parikh et al. while in Amit et al benign lesion were higher. In our study 70% were of tubercular lymphadenitis, which was comparable with vanisri et al had 58.3% , rest study had lower incidence of tuberculous lymphadenitis as compare with our study. In present study, 6% in suppurative lymphadenitis, which was comparable with Desmukh et al. had 6.81%. In the present study among Benign lesions cytologically diagnosed, maximum number of cases, were 12% in reactive lymphadenitis group In the study of desmukh et al. 29.5% were in the reactive lymphadenitis group.

Among neoplastic lesions, in our study the most common was lymphoma 5% followed by metastatic deposit in 3%. Similar results were seen in above mentioned studies. Shenoy et al. and Vanisri et al. also did not found any other opportunistic infection. Bates et al. Noted one case of *Histoplasma* and one case of *Cryptococcus*. Satyanarayan et al. noted one case of *Cryptococcus* and one case of *Rhodotorula*. Tuberculous lymphadenitis was predominant cytopathological diagnosis in the present study constituting 70% cases. Studies conducted by Shenoy et al (50%) in Mangalore, Guru et al<sup>1</sup> (41.55%) in Chadigarh and Amit et al. 114 (38%) in Rajkot also observed tuberculous lymphadenitis as a common lymph node lesion. However studies conducted in California by Bottles et al<sup>11</sup> (17%) and in Europe by Reid et al<sup>12</sup> (15%) and Martin-Bates et al<sup>11</sup> (22%) demonstrated lower number of cases in comparison with recent study. In total 70 cases of tuberculous lymphadenitis on FNAC, Ziehl Neelsen stain was done. Out of which 50(71.42%) cases were positive for AFB and 20(28.53%) were negative. While in Amit et al. 53(46.33%) cases were positive for AFB and 61(53.51%) were negative. And rate of AFB positivity was reported by Ahmed et al<sup>15</sup> (46.4%) in 2005. Metre and Geeta Jayaram (1987) demonstrated acid fast bacilli in 56.4% of tuberculous lymphadenitis.

In present study among tuberculous lymphadenitis patients CD4 range was 49-640 and median CD4 count was 210 while in Shobhana et al. CD4 range was 143-422 while median CD4 count was 212 which comparable with present study. There was low CD4 count range 66-145 in lymphoma patient, while in Shobhana et al. two cases had lymphoma having CD4 count 79 and 113 respectively which correlates with present study. CD4 count more than 500 cells/ $\mu$ L was seen in 10 (6%) cases of tuberculous lymphadenitis in our study. Patients with tubercular infection had CD4 count between 200- 499 cells/  $\mu$ L in 30 (42%) cases and less than 200 in 30 (42%) cases

which was correlates with Desmukh et al. shows 55% and 45% accordingly. In total 70 cases of tuberculous lymphadenitis 52.85 % had Epitheloid granuloma with caseation, 20% had granuloma without caseation, 27.14% had only caseous necrosis, which is comparable with Rajshekharan et al. subsequently 37.5%,37.5% and 25% and Desmukh et al. subsequently 50%, 15% and 35%.

In total 70 cases of tuberculous lymphadenitis CD4 counts were available in all 70 cases. The mean CD4 cell count was 210. Similar findings were recorded by Sobhana et al<sup>17</sup> with median CD4 count 212, Amit et al. 261.8 .Guru et al. <sup>5</sup> recorded mean CD4 count of 123.8 in tuberculous lymphadenitis. In total 12 cases of reactive lymphadenitis CD4 counts were available in all 12 cases. The mean CD4 count in our study was 313 which is comparable with Guru et al<sup>5</sup> with CD4 count 311.4, Amit et al. 285.4.Shobhana et al.<sup>17</sup> found slightly higher value(672)of median CD4 count for reactive lymphadenitis.

In metastatic malignancies CD4 count available in all 3 cases. The mean CD4 count was 105. Guru et al.<sup>5</sup> recorded mean CD4 count of 105. Similarly in Lymphoma lesions CD4 count available in all 5 cases and the mean CD4 count was 98 which is comparable with Amit et al. 153.5. Sobhana et al.<sup>17</sup> found CD4 count in only two cases of NHL which were of 79 & 113.

## CONCLUSION

The maximum numbers of cases were in the age group of 31-40yrs. The Range of age of patients was from 4 month-70 years with median age of 35.

The incidence of lymphadenopathy decreased with advancing age after 50 years.

The male to female ratio was approx 2:1.

Most common region involved in study was cervical 77% followed by axillary region 18 % while both region 5 %

Incidence of infectious lesion 76% was higher in present study While in 8 cases malignancy were found.

Maximum number of patients were having tuberculous lymphadenitis (70%) followed by reactive lymphadenitis were found in 12 patients followed by acute suppurative lymphadenitis in 6 patients. While in lymphoma all 5 cases and metasatic deposit 3 cases all having cervical lymphadenopathy Also there had 2 cases of benign lymphoepithelial lesion and each case of keratanious cyst and cystic swelling found on cytology.

We found 5 cases of lymphoma each showing cytological features of anaplastic large cell lymphoma, large cell lymphoma, non hodgkins lymphoma, hodgkins lymphoma, & diffuse large cell lymphoma having CD4 count of 145, 104, 78, 66, & 98 subsequently. Out of them Anaplastic large cell lympho-

ma, diffuse large cell lymphoma & hodgkins lymphoma were confirmed histopathologically. While in other 2 cases histopathology was not available.

Maximum number of malignant cases were found in 3<sup>rd</sup> decade.

Out of 100 cases, CD4 count  $\geq 500$  seen in 16 patients, 200-499 in 42 patients, while  $< 200$  were seen in 42 patients.

In tuberculous lymphadenitis(70 cases), the median CD4 count was 210 (49-640); acute suppurative lymphadenitis(6 cases), the median CD4 count was 338 (116-520), reactive lymphnode (12 cases), the median CD4 count was 313 (119-670), lymphoma (5 cases), the median CD4 count was 98 (66-145), metastatic deposits (3 cases), median CD4 count was 105 (78-360) & benign lymphoepithelial lesion (2 cases), the median CD4 count was 641.5 .

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