



THE STUDY OF CO-RELATION BETWEEN CD4 COUNTS & LYMPH NODE FINE NEEDLE ASPIRATION CYTOLOGY IN HIV POSITIVE PATIENTS AT TERTIARY HEALTH CARE INSTITUTE IN WESTERN MAHARASHTRA

Pathology

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ABSTRACT

**Background:** Lymphadenopathy in HIV infection is very common. Apart from other causes of lymphadenopathy, HIV infection itself may produce persistent generalized lymphadenopathy. The present study demonstrated the utility of Lymph Node (LN) cytology in the diagnosis and segregation of lymphadenopathy cases in HIV positive cases to aid clinical management.

**Aims:**

1. To find out various pathological changes in lymph node by fine needle aspiration cytology (FNAC) in HIV positive patients.
2. To find various types of lymph node lesions according to CD4 count in HIV positive patients.
3. To study co-relation of CD4 count with lymph node Fine needle Aspiration Cytology in HIV positive patients.

**Materials & Methods:** Present prospective study was conducted in the department of Pathology, over a period of one & half years. Study included HIV positive patients referred for FNAC of enlarged LNs. Patients below 15 years of age were excluded.

Statistical Analysis used: Statistical analysis was carried out for difference in CD4 counts in five subgroups of reactive lymphadenitis, acute inflammatory lesion, granulomatous lymphadenitis, TB lymphadenitis and lymphoma by Chi Square Test

**Results:** Total 118 aspirations were obtained, out of which 115 (97.5%) had HIV-1 infection. Cervical LN was most commonly involved in 57 (48.3%) cases. Out of total 118 cases, 50 (42%) had tuberculous (TB) lymphadenopathy, followed by reactive lymphadenitis 35 (29.6%). Analysis of difference in CD4 counts in five subgroups of reactive lymphadenitis, acute inflammatory lesion, granulomatous lymphadenitis, TB lymphadenitis and lymphoma was statistically significant ( $p < 0.05$ ). Out of 118 patients majority 51 (43.2%) patients had CD4 values  $> 500$  cells/ $\mu$ l.

**Conclusion:** FNAC is the primary and safe investigative procedure for lesions of the LNs in HIV positive patients and in many cases; it obviates the need for excision, guides subsequent therapy or observation.

KEYWORDS

HIV, Lymph node, CD4 count, FNAC

INTRODUCTION:

AIDS, the Acquired Immunodeficiency Syndrome (sometimes called "Slim disease") is a fatal illness caused by retrovirus known as the Human Immunodeficiency Virus (HIV) which breaks down the body's immune system, leaving the victim vulnerable to threatening opportunistic infections (OI), neurological disorders or unusual malignancies. Recognized as an emerging disease only in early 1980s, AIDS has rapidly established itself throughout the world and is likely to endure and persist well into the 21<sup>st</sup> century<sup>1</sup>.

Though India is the country with low HIV prevalence, it has third largest number of people living with HIV/AIDS<sup>2</sup>. LNs are commonly involved during all the stages of infection and are one of the earliest signs in HIV infected patients. Lymphadenopathy may also be a manifestation of opportunistic infections (OIs) and lymphoid malignancy developing in immunocompromised individuals. Clinical examination and radiological investigations on their own have diagnostic limitations.

As the HIV virus primarily infects lymphocytes, CD4 lymphocytes are mainly affected. These helper cells initiate body's response to invading micro-organisms such as viruses. CD4 cells are the host cells that aid HIV in replication. HIV attaches to the CD4 cells allowing the virus to enter and infect the CD4 cells, damaging them in process. The fewer functioning CD4 cells, the weaker the immune system and therefore the more vulnerable a person is to infections and illnesses. In a healthy adult, normal CD4 count is typically 600- 1200/cmm of blood. In HIV positive patient CD4 count between 600 – 350 is considered 'very good'. A CD4 count  $< 200$ , the immune system is severely weakened and the HIV positive person is at greater risk of OIs<sup>3</sup>. So these CD4 cells are used to assess immune status, susceptibility to OIs, need for ART and defining AIDS.

In developing countries like India, with huge population and socioeconomic constraints, there is great need for simple investigative technique for HIV infected lymphadenopathy cases. FNAC is a simple

and rapid diagnostic technique. Due to early availability of results, simplicity, minimal trauma and minimum complications, FNAC is now considered as valuable diagnostic aid and is gaining popularity. The role of FNAC in lymphadenopathy is well known. With the increased availability of equipments to perform CD4 counts and knowledge that CD4 cells are primary target of HIV, CD4 count estimation became standard measure of immunodeficiency in HIV infected patients. Relative ease of CD4 count also lead to its advocacy in treatment guidelines when to start, stop and change ART and deciding when to initiate prophylaxis for OIs. This is in spite the fact that CD4 count does not always correlate with functional immunity. Some patients with normal CD4 are susceptible to OIs while patients with decreased CD4 do not seem susceptible to OIs.

Present study was performed to evaluate the role of FNAC as a cytological investigative technique in diagnosis of various cytological patterns of lesions in HIV lymphadenopathy and to correlate cytological findings with CD4 count of the patient.

MATERIALS AND METHODS:

Present prospective study was conducted in the department of Pathology on outpatient department (OPD) basis, from January 2010 to June 2011, over a period of one and half years. Study cases included the HIV positive patients referred to pathology OPD from ART centre for FNAC of enlarged LNs. During the study period, 118 LN fine needle aspirates were obtained from known 118 HIV positive patients after getting informed consent. Study included patients in reproductive age group and the patients below 15 years of age were excluded. All the patients got their baseline CBC and recent CD4 count. Detailed clinical history was obtained as per the proforma. The patients were subjected for FNAC under all aseptic and universal safety precautions. Careful removal and disposal of syringes and needles were done. For safety precautions, multiple passes through nodes was avoided. Enlarged LNs were examined completely including the site, size, consistency, mobility and then were aspirated. Adequacy of specimen was checked at the time of aspiration. All the smears were stained by H

& E , Papanicolaou stain and Ziehl Neelson (ZN) stain for acid fast bacilli (AFB). Biopsy was taken for the confirmation in a case of lymphoma diagnosed on FNAC.

**Smear patterns observed in the study:**

Pattern of smears in all the cases were viewed in background of clinical details. All smears screened under low power and high power magnification of microscope and classified into following categories:

1. Tuberculous (TB) lymphadenitis (**Image 2**)
2. Reactive lymphadenitis (**Image 1**)
3. Granulomatous lymphadenitis
4. Acute inflammatory lesion
5. Positive for malignant cells (neoplastic lesion)

HIV status of patients was confirmed according to NACO guidelines<sup>4</sup>.

**CD 4 Count Measurement:**

CD 4 count of each patient was done with the help of Partec Cyflow Counter

**Statistics:**

Statistical analysis was carried out for difference in CD4 counts in five subgroups of reactive lymphadenitis, acute inflammatory lesion, granulomatous lymphadenitis, TB lymphadenitis and lymphoma by Chi Square Test.

**RESULTS:**

**Age wise distribution of cases**

In our study we have excluded patients below 15 yrs of age and we subdivided cases into groups as <20 yrs, 21-30 yrs, 31-40 yrs and >40 yrs.

We found 60 (50.8 %) patients were in 31-40 yrs age, followed by 21-30 yrs 48(40.7%)

**Sex wise distribution of cases**

Sex distribution showed male predominance. Out of 118 cases, 86 (72.9 %) were males and 32 (27.1 %) were females. Male: female ratio was 2.6:1

**Distribution of cases according to HIV species**

Majority of the patients 115 (97.5 %) had HIV -1 infection while 2 cases of HIV -2 infection (1.7 %) and only one case of dual infection i.e. with HIV-1 & HIV-2 was noted.

**Clinical categories of cases according to CDC classification**

Out of 118 cases, 51 (43.2 %) were category C patients according to CDC classification, followed by 38 (32.2 %) cases in category B, 29 (24.6 %) were in category A (**Table 1**)

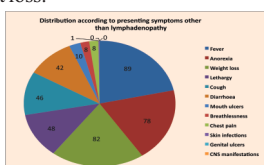
**Table1. Clinical categories of cases according to CDC Classification**

CLINICAL CATEGORIES	NO. OF PATIENTS		TOTAL (N=118)	PERCENTAGE (%)
	Males	Females		
A Acute HIV/ Asymptomatic/PGL	23	6	29	24.6
B Symptomatic Not A or C	27	11	38	32.2
C AIDS indicator conditions	36	15	51	43.2

**Distribution according to presenting symptoms other than lymphadenopathy**

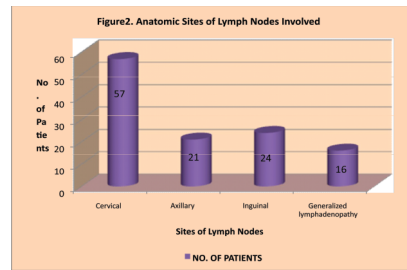
The important presenting clinical features of these patients are depicted in (**Figure1.**)

Present study included cases presenting with lymphadenopathy. Apart from lymphadenopathy, fever was most common presenting symptom followed by weight loss.



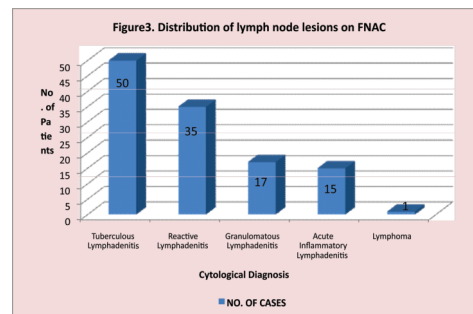
**Anatomic sites of LNs involved (Figure 2.)**

In our study, cervical group of lymph nodes were affected in 57 (48.3 %) of cases, followed by 24 cases of inguinal (20.3 %) lymph nodes.



**Distribution of LN lesions on FNAC(Figure3.)**

TB lymphadenitis was the most common finding in 50 cases (42.4 %) followed by 35 (29.6 %) cases of reactive lymphadenitis. 17 (14.4 %) showed granulomas on smear but these cases were AFB negative. These cases were called granulomatous lymphadenitis. No specific etiology could be determined in these cases. 15 (12.8 %) cases showed acute inflammatory changes, while one case was positive for malignant cells, diagnosed as lymphoma (**Image 4 & 5**).



**Cd4 values in study population (Table 2.)**

Out of total 118 study cases, 51 (43.22 %) cases had CD4 value more than 500 cells/ µl and 28 (23.7 %) had CD4 less than 200 cells/ µl.

**Table2. CD4 values in study population**

Cd4 COUNT (CELLS /µL)	NO. OF CASES	PERCENTAGE (%)
≥ 500	51	43.2
200 – 499	39	33.1
< 200	28	23.7
<b>Total</b>	<b>118</b>	<b>100</b>

**Lymph node cytological smear pattern and CD4 count (Table 3.)**

The 5 cytological patterns which are described earlier were correlated with CD4 counts of patients and are depicted in **Table 3.**

**Table3. Lymph node cytological smear pattern and CD4 count**

CD4 Range ( Cells / µl)	Mean Value Of CD4 Count (µl)	Median Value Of CD4 Count (µl)	Cytological Diagnosis By FNAC	No. Of Cases	Percentage (%)
65 – 595	243.82	200	Tuberculous Lymphadenitis	50	42.4
153 – 906	620.79	628	Reactive Lymphadenitis	35	29.6
200 – 770	446.43	412	Granulomatous Lymphadenitis	17	14.4
329 – 946	641.1	625	Acute Inflammatory Lesion	15	12.8
34	--	--	Lymphoma	01	0.8

**Co-relation of CD4 count with cytological diagnosis(Table4.)**

Analysis of difference in CD4 counts in five subgroups of was statistically significant (p < 0.05)

**Table4. Co-relation of CD4 count with cytological diagnosis**

CD4 Cell Count (Cells / $\mu$ l)	TB Lymph adenitis	Reactive Lymphadenitis	Granulomatous Lymphadenitis	Acute Inflammatory Lesion	Lymphoma	Total
<200	26 (92.8%)	1 (3.6 %)	0	0	1 (3.6%)	28
200 -499	21 (53.9 %)	5 (12.8 %)	11 (28.2 %)	2 (5.1 %)	0	39
$\geq$ 500	3 (5.9 %)	29 (56.9 %)	6 (11.8 %)	13 (25.4 %)	0	51
<b>TOTAL</b>	<b>50</b>	<b>35</b>	<b>17</b>	<b>15</b>	<b>1</b>	<b>118</b>

Chi square value= 62.99, d.f. =1, p<0.05 (significant)

#### DISCUSSION:

In the present study, 118 cases were included. All the cases presented with lymphadenopathy and proved to be HIV positive as per NACO guidelines<sup>4</sup>. Lymphoid tissues are the prime targets in AIDS, affected by HIV infection as well as by OIs and neoplasms<sup>5</sup>. Regardless of portal of entry of HIV, they are major anatomic sites for establishment and propagation of HIV infection<sup>6</sup>.

The present study demonstrated the utility of LN cytology in the diagnosis and segregation of lymphadenopathy cases in HIV positive cases to aid clinical management.

#### Comparison for age

Study included patients aged > 15 years. Patient's age ranged from 21 – 45 years. 60 (50.8 %) patients were in age group 31 –40 years.

In a study conducted by Bottles et al (1988)<sup>6</sup> age of HIV positive patients ranged from 18 –52 years while in a study by Bates et al (1993)<sup>7</sup> age of patients ranged from 19- 72 years.

Shobhana et al (2002)<sup>8</sup> studied 54 HIV positive patients with lymphadenopathy for FNAC and most common affected age group was 21-30 years. Vanisri et al (2008)<sup>9</sup> found maximum number of cases in age group 21 – 30 years, followed by 31-40 years. Kumarguru et al (2009)<sup>10</sup> studied 231 HIV positive cases referred to pathology department for FNAC of lymph nodes and majority of the cases 110 In a study conducted by Bottles et al (1988)<sup>6</sup> age of HIV positive patients

ranged from 18 – 52 years while in a study by Bates et al (1993)<sup>7</sup> age of patients ranged from 19- 72 years.

#### Comparison of Sex Distribution

In present study, sex distribution of study cases revealed male predominance. Out of 118 patients, 86 (72.9 %) were males and 32 (27.1 %) were females with male: female = 2.6:1.

In study conducted by Kumarguru et al (2009)<sup>10</sup> males were affected more than females with a male: female ratio 2.3:1. In a study performed by Shenoy et al (2002)<sup>11</sup> male: female ratio was 5:1.

#### HIV serotype

Out of 118 patients, 115 (97.5 %) had evidence of HIV -1 infection, 2 (1.7 %) cases had HIV -2 infection while only one case had dual infection (HIV-1 & 2). In a study by Shobhana et al (2002)<sup>8</sup> out of 54 patients, all the patients had evidence of HIV-1 infection excepting one case of dual infection (HIV – 1& 2).

#### Comparison for LN site

In present study cervical LNs were most commonly affected. Out of 118 patients, 57 (48.3 %) had cervical lymphadenopathy. In a study conducted by Bottles et al (1988)<sup>6</sup> cervical group of s was found to be most commonly affected site. In a study by Bates et al (1993)<sup>7</sup>, cervical LNs were most affected site. In a study by Jayaram et al (2000)<sup>12</sup> out of 39 patients, 26 had cervical lymphadenopathy. Vanisri et al (2008)<sup>9</sup> found cervical LN as the most common site. However; Satyanarayna et al (2002)<sup>13</sup> reported axillary node involvement being more common site in their study.

#### Comparison of pattern of lymphadenopathy with different authors

Author & year	Total no. of cases	Reactive %	Tuberculous %	Granulomatous %	Acute inflammatory lesion %	Lymphoma %	Suppurative %	Cryptococcal %	Kaposi's sarcoma %	Other %
Bottles et al <sup>6</sup> (1988)	121	50	17	--	--	20	--	--	10	3
Strigle et al <sup>14</sup> (1992)	396	35	--	--	---	12	12.5	--	4	--
Bates et al <sup>7</sup> (1993)	27	41	22	--	--	7	--	--	15	23
Grossl et al <sup>15</sup> (1997)	59	61	--	6.7	8.4	6.7	--	--	--	16.9
Reid et al <sup>16</sup> (1998)	65	51	15	--	--	9	--	--	2	23
Jayaram et al <sup>12</sup> (2000)	39	24	53.8	2.5	--	2.5	--	2.5	--	12.8
Saikia et al <sup>17</sup> (2001)	25	40	32	--	--	4	10	--	--	--
Shobhna et al <sup>8</sup> (2002)	54	55.5	41	--	--	3.7	--	--	--	--
Shenoy et al <sup>11</sup> (2002)	48	36	50	--	--	10	--	--	--	4
Satyanarayana et al <sup>13</sup> (2002)	196	42.3	34.2	--	--	2.6	23.5	--	--	--
Sujata nayak et al <sup>18</sup> (2003)	32	31.5	50	--	--	6.25	--	--	--	15.65
Vanisri et al <sup>9</sup> (2008)	36	36.1	58.3	--	--	2.7	2.7	--	--	--
Kumarguru et Al <sup>10</sup> (2009)	231	46.3 2	41.5 5	7.3	--	1.73	1.29	0.43	--	--
<b>Present study (2011)</b>	<b>118</b>	<b>29.6</b>	<b>42.4</b>	<b>14.4</b>	<b>12.3</b>	<b>0.8</b>	<b>--</b>	<b>--</b>	<b>--</b>	<b>--</b>

### Co-relation of CD4 count with lymph node FNAC in comparison to others

Pattern of Lymphadenopathy	Study	Median of CD4 count cells/ $\mu$ l
Reactive hyperplasia	Shobhana et al <sup>8</sup> (2002)	672
	<b>Present study (2011)</b>	<b>628</b>
Tuberculous lymphadenitis	Shobhana et al <sup>8</sup> (2002)	212
	<b>Present study (2011)</b>	<b>200</b>
Lymphoma	Shobhana et al <sup>8</sup> (2002)	<100
	<b>Present study (2011)</b>	<b>34</b>

Analysis of difference in CD4 counts in five subgroups of reactive lymphadenitis, acute inflammatory lesion, granulomatous lymphadenitis, TB lymphadenitis and lymphoma was statistically significant ( $p < 0.05$ ). General downward trend in median CD4 count was observed in subgroups of reactive lymphadenitis (median 628 cells/  $\mu$ l), acute inflammatory lesion (median 625 cells/  $\mu$ l), granulomatous lymphadenitis (median 412 cells/  $\mu$ l), TB lymphadenitis (median 200 cells/  $\mu$ l) and lymphoma (CD4 count 34 cells/  $\mu$ l).

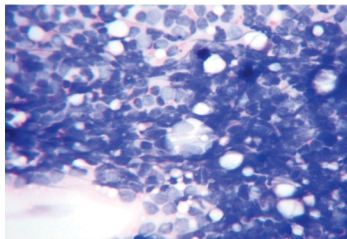
The present study concluded the fact that TB lymphadenitis, reactive lymphadenitis and granulomatous lymphadenitis are most common causes of lymphadenopathy in HIV positive patients. However in granulomatous condition no etiology other than tuberculosis could be determined in our study.

### CONCLUSION:

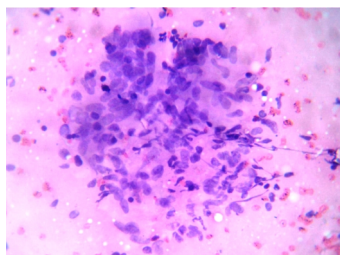
FNAC is the primary and safe investigative procedure for lesions of the LNs in HIV positive patients and in many cases; it obviates the need for excision, guides subsequent therapy or observation. Most OIs can be identified and high grade lymphomas can be diagnosed. The method is accurate, cost effective and acceptable to patients.

In developing countries, TB is the most common OI in HIV infected people. Immunosuppression due to HIV is the most common predisposing factor for the development of TB. It was observed in present study. The LN cytology is useful for segregating lymphadenopathy cases that needs further evaluation. Granulomatous lymphadenitis needs further work up. Neoplastic lesions need biopsy & Immunohistochemistry for further typing. It is a valuable tool for identification of opportunistic infections, neoplastic lesions and non-neoplastic lesions. Correlation of lesions with CD4+T lymphocyte counts provides information about immune status and stage of the disease. General downward trend in median CD4 count was observed in subgroups of reactive lymphadenitis, acute inflammatory lesion, granulomatous lymphadenitis, TB lymphadenitis and lymphoma. In addition, co-relating AFB staining pattern in TB lymphadenitis reflects disease activity.

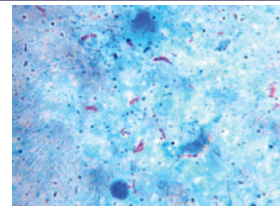
### Images:



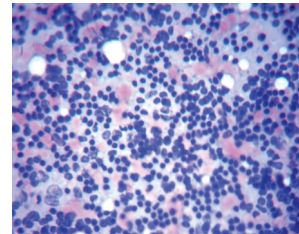
**Image1. Reactive Lymphadenitis - FNAC showing mixed population of Centroblasts, Centrocytes & Small Lymphocytes (HE 400X)**



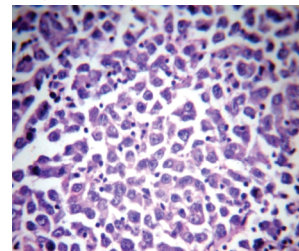
**Image2. Tuberculous Lymphadenitis – FNAC showing Epitheloid cell granulomas (HE 400X)**



**Image 3. Acid fast bacilli – FNAC showing plenty pink rod shaped AFB, Grade 3+ (ZN, 1000X)**



**Image4. Non- Hodgkin's Lymphoma – FNAC diffuse monotonous population of large lymphoid cells with vesicular nuclei & prominent nucleoli (HE, 400X)**



**Image5. Non- Hodgkin's Lymphoma– HPE showing diffuse arrangement of lymphoid cells with vesicular nuclei & prominent central nucleolus and amphophilic cytoplasm. (HE, 400X)**

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