



## FINE NEEDLE ASPIRATION CYTOLOGY FINDINGS IN LYMPHNODE OF HIV PATIENTS

R. Sindhuja

Assistant Professor Department Of Pathology Tirunelveli Medical College Tirunelveli

Dr. D. Rajeswari  
Thivya\*Assistant Professor Department Of Pathology Chettinad Hospital And Research  
Institute \*Corresponding Author

**ABSTRACT** **BACKGROUND:** Acquired Immuno Deficiency Syndrome caused by Human Immuno Deficiency Virus infection is one of the major public health problem. Extra pulmonary tuberculosis in the form of node involvement correlates well with advanced HIV disease and low CD4 count. Fine Needle aspiration Cytology has a well known role in the evaluation of lymphadenopathy in HIV- infected cases.

**AIM**

- 1.To find the various FNAC findings in lymph node of HIV patients.
- 2.To correlate the various FNAC findings with CD4 count.
- 3.To correlate the various FNAC findings with Acid Fast Bacilli stain

**CONCLUSION:** Fine Needle Aspiration Cytology is an useful tool in identifying the causes of lymphadenopathy . Correlation of Fine Needle Aspiration Cytology finding with CD4 count and AFB staining helps the clinician to access the immune status of the affected individual.

**KEYWORDS :** HIV, FNAC, lymphnode, CD4 COUNT, AFB SCORING**INTRODUCTION**

Acquired Immuno Deficiency Syndrome caused by Human Immuno Deficiency Virus infection is one of the major public health problem. It is a major pandemic in South East Asian countries.<sup>1</sup> AIDS was first recognized, in 1981 when Pneumocystis jirovecii pneumonia, is reported in five healthy homosexual men and Kaposi's sarcoma in 26 healthy homosexual men.<sup>2</sup> HIV affects primarily the CD4 (cluster of differentiation) T lymphocytes, which acts as the major target for them. So as the disease progresses, the CD4 count progressively declines leading on to defect in the cell mediated immunity. Lymph node involvement is the earlier sign to occur in HIV infected individuals as the virus primarily affects the lymphocytes. Tuberculosis is the most common opportunistic infection in HIV/ AIDS infection. Infected individuals with good immune response and high CD4 count will have typical symptoms and smear positive for AFB in sputum. In individuals with severe immunosuppression, the CD4 counts are low and the clinical and radiological pictures are atypical and presents with extra pulmonary tuberculosis.<sup>3</sup> Extra pulmonary tuberculosis in the form of cervical node involvement correlates well with advanced HIV disease and low CD4 count.<sup>4</sup> There is need for simple investigating procedure for lymphadenopathy in HIV infected individuals in developing countries like India.<sup>5</sup> Fine Needle aspiration Cytology has a well known role in the evaluation of lymphadenopathy in HIV- infected cases.<sup>6</sup> FNAC is a cheap, painless, noninvasive, outpatient procedure with less morbidity. This helps in effectively diagnosing the cause of lymphadenopathy in HIV infected cases and helps in the initiation of treatment at an earlier stage.

**METHODOLOGY**

This study includes a total of 50 fine needle aspirates obtained prospectively from patients who came to our Pathology department. They are reactive for HIV by ELISA with lymph node enlargement during the study period of June 2012- May 2013. Patients age, clinical history, CD4 counts were recorded.

Two smears are done simultaneously. One is used for H&E staining and the other is heat fixed and used for ziehl neelson staining for acid fast bacilli. Acid fast bacilli appears pink. AFB scoring was done simultaneously.

**RESULTS**

Our study is a prospective study for a period of one year from June 2012- May 2013. 50 fine needle aspirates obtained prospectively from patients who came to our Pathology department who are reactive for HIV by ELISA with lymph node enlargement were examined.

Among the 50 cases, the most common age group affected was 41-50 years (40%). Males (68%) are more commonly affected than females (32%). The male to female ratio was 2.12:1.

Among the 50 cases 38 cases (76%) had involvement of single group of lymph node and 12 cases (24%) had involvement of multiple sites of lymph node.

The most common site of involvement was cervical group constituting 64% (32cases) . The next predominant site involved was the submandibular and axillary node constituting 12% each.

Tuberculous lymphadenitis was the most common pattern observed in Fine Needle Aspiration Cytology. This pattern was seen in 37 (74%) cases. The next common pattern observed was reactive lymphadenitis which was seen in five (10%) cases. Table 1

**Table 1 - Fnac Findings In Lymph Node Of Hiv Patients**

FNAC FINDING	NUMBER	%
TUBERCULOSIS	37	74
REACTIVE	5	10
SUPPURATIVE	2	4
METASTASIS	1	2
LYMPHOMA	1	2
BLOOD COMPONENTS	4	8
TOTAL	50	100

The most common age group affected by tuberculosis was 41-50 years constituting 20 cases (54.05%).Males (64.86%) are more commonly affected than females (35.14%). The male to female ratio was 1.85:1.

Among the 37 cases of tuberculous lymphadenitis, the most common pattern observed in FNAC was caseating granulomatous lymphadenitis (45.94%), followed by caseating lymphadenitis and granulomatous lymphadenitis constituting 35.14% and 18.92% respectively. Table 2

**Table 2 - Patterns Of Tuberculosis In Fnac**

PATTERNS	NUMBER	%
CASEATING LYMPHADENITIS	13	35.14
CASEATING GRANULOMATOUS LYMPHADENITIS	17	45.94
GRANULOMATOUS LYMPHADENITIS	7	18.92
TOTAL	37	100

The mean CD4 count of the patient in this study was 149.2 cells/  $\mu$ l. Among the cases the least CD4 count was 38 cells /  $\mu$ l and the highest CD4 count was 323 cells/  $\mu$ l.

The mean CD4 count in case of caseating lymphadenitis was 71.77 cells/ $\mu$ l. The mean CD4 count in case of caseating granulomatous lymphadenitis was 145 cells/ $\mu$ l. The mean CD4 count in case of granulomatous lymphadenitis was 180.71 cells/ $\mu$ l.

Grade 2+ was the predominant AFB grading in tuberculous lesions. It was seen in 21 patients (56.76%). This was followed by grade 1+ and grade 3+ constituting 14 patients (37.84%) and two patients (5.4%) respectively.

Grade 2+ was the predominant pattern observed in all the tuberculous lesions. It was seen in 21 cases. In caseating lymphadenitis the predominant grade was grade 2+, constituting eleven cases (52.38%). In caseating granulomatous lesion, the predominant grade was grade 2+, constituting ten cases (47.62%). In granulomatous lymphadenitis, all the cases show grade 1+ AFB staining pattern.

**Table 3 - Correlation Of Various Tuberculous Patterns In Fnac With Mean Cd4 Count And Afb Grading**

PATTERNS OF TUBERCULOSIS	Number of cases	%	AFB grading Z			Mean CD4 count
			Grade	Number of cases	%	
Smears with epithelioid granuloma alone	7	18.92	1+	7	100	180.71
Smears with caseous necrosis and epithelioid granuloma	17	45.94	1+	7	41.18	167.28
			2+	10	58.82	129.4
Smears with caseous necrosis alone	13	35.14	2+	11	84.62	71.91
			3+	2	15.38	38
TOTAL	37	100		37		

It is observed from the table 3 that, the mean CD4 count decreased progressively with increase in the grading of AFB and appearance of necrosis.

**DISCUSSION**

The present study demonstrated the utility of Fine Needle Aspiration Cytology of lymph node in the diagnosing and segregating the causes of lymphadenopathy in HIV positive patients to aid in the management.

Among the cases, the youngest age was 3 years and oldest was 64 years. The commonest age group affected was fourth decade of life (40%) followed by third decade (36%). This was similar to the study observed by Parikh U.R. et al.<sup>7</sup> The age group most commonly affected was fourth decade (32.5%) followed by third decade (22.5%).

The male to female ratio was 2.12:1. This was similar to the study done by Shobana et al, in which the male to female ratio was 2.85:1<sup>1</sup>.

In the present study, cervical group of lymph node was the most common site involved. This was similar to the study observed by Bates et al,<sup>8</sup> Parikh U.R. et al,<sup>7</sup> Vanisri et al<sup>9</sup> and Shenoy et al.<sup>10</sup> Sathyanarayana et al<sup>11</sup> had found axillary lymph node to be the most common site involved.

The causes of lymphadenopathy in our study were:

- Tuberculosis – 37 (74%)
- Reactive - 5 (10%)
- Suppurative - 2 (4%)
- Metastatic deposits- 1 (2%)
- Lymphoma - 1 (2%)
- Blood and blood components – 4 (2%)

In the present study (74%), as well as in the study performed by Parikh U.R. et al<sup>7</sup> (40.54%) and Vanisri et al<sup>9</sup> (58.33%) infections caused by Mycobacterium tuberculosis was the most common cause in HIV patients with lymphadenopathy. This is because of the increased prevalence of tuberculosis in our country and also because of the defective immune function in patients with HIV. Guru et al<sup>12</sup> (46.32%) and Shobana et al<sup>1</sup> (55.50%) had found reactive lymphadenitis being the commonest cause of lymphadenopathy in HIV patients.

The difference can be because of the difference in patient selection, socioeconomic status of the patients and CD4 counts. Most of the patients in our study belong to low socio economic group and tuberculosis is one of the commonest infection seen in them. The diagnosis of tuberculous lymphadenitis was made only when the smears were positive for Acid Fast Bacilli stain. Guru et al<sup>12</sup> had also used the same criteria to diagnose tuberculous lymphadenitis.

The smears of tuberculous lymphadenitis was grouped into three

categories as fig 2,3

- Smears with caseous necrosis alone (Caseating Lymphadenitis) – 13%
- Smears with caseous necrosis and epithelioid cell granuloma (Caseating Granulomatous Lymphadenitis)-17%
- Smear showing epithelioid granuloma alone. (Granulomatous Lymphadenitis) -7%

Smears with caseous necrosis and epithelioid granuloma constitutes the predominant pattern with 17 cases (45.94%), followed by smears with caseous necrosis alone with 13 cases (35.14%) and epithelioid granuloma alone 7 cases (18.92%). This was similar to the study observed by Parikh U.R. et al,<sup>7</sup> Vanisri et al<sup>9</sup> and Guru et al<sup>12</sup>. Reactive lymphadenitis was the second most common lesion observed in five cases (10%). This was similar to the study observed by Vanisri et al.<sup>9</sup> In their study also reactive lymphadenitis (36.10%) was the second common lesion observed.

Suppurative lymphadenitis was observed in 2 (4%) of cases. This was similar to the study observed by Vanisri et al (2.70%)<sup>9</sup> and Guru et al (1.29%).<sup>12</sup>

One case (2%) was diagnosed as lymphoma. This was similar to the study observed by Parikh U.R. et al (2.70%)<sup>7</sup> and Vanisri et al (2.70%)<sup>10</sup>.

In our study one case (2%) was diagnosed as metastatic deposits. (Fig 4). The patient was found to have deposits from squamous cell carcinoma of eyelid. Guru et al<sup>12</sup> had found three cases (1.23%) of metastatic deposits. All these cases have metastatic adenocarcinomatous deposits. Parikh U.R et al<sup>7</sup> and Vanisri et al<sup>10</sup> had not found any cases of metastatic deposits in their study.

Four cases (8%) on repeated aspiration yielded only blood and blood components in our study. This is because of the very small size of the node ranging between 0.3-0.5 cm

In our study, patients with tuberculous lymphadenitis had CD4 count range between 38-198 cells / microlitre and the mean CD4 count was 126.04 cells/ micro litre. This was similar to the study observed by Ajay Jarya et al who had observed that Tuberculous lymphadenitis was common in patients with CD4 count <200 cells/microlitre.<sup>13</sup>

Non hodgkins lymphoma was diagnosed in a single case with CD4 count of 68 cells/microlitre. Shobana et al in their study had diagnosed two cases of Non hodgkins lymphoma with CD4 count 79-113 cells/microlitre.

Metastatic deposits was observed in one case with CD4 count of 156 cells/microlitre. This was similar to the study observed by Grossl et al<sup>14</sup> with a mean CD4 count of 105 cells/microlitre.

In the present study, mean CD4 count was highest in case of reactive lymphadenitis (253.4 cells/microlitre). The CD4 count ranges between 218- 287 cells/microlitre. This was similar to the study observed by Guru et al<sup>12</sup> with the highest mean CD4 count of 311.40 cells / microlitre in case of reactive lymphadenitis when compared to other lesions.

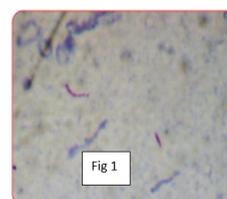
AFB grading was done based on the staining pattern as observed by Kumar et al.<sup>15</sup> Fig 1,2,3

Grade 1 – Acid Fast Bacilli was found in the smear after a careful search.

Grade 2 – Acid Fast Bacilli were found as singly scattered

Grade 3 – Acid Fast Bacilli were found in the smears in large numbers. They are in faggots or as singly scattered.

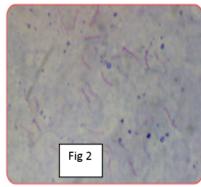
AFB staining show singly scattered bacilli (100X)



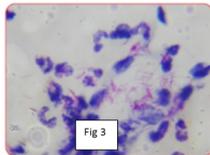
Grade 2+ was the most common pattern observed in 21 (56.76%) of

cases. This was similar to the pattern as observed by Guru et al.<sup>12</sup> This is in contrast to the study as observed by Kumar et al in whom the predominant pattern observed was grade 1+.<sup>15</sup> This difference is because the aspirates in the study done by Kumar et al included both HIV and non HIV individuals. The non HIV individuals are immunocompetent.

AFB staining show sparsely scattered bacilli throughout the smear (100X)



AFB staining show large number of bacilli in clusters and faggots (100X)



Correlation of AFB grading with various patterns of tuberculous lymphadenitis had showed that smears with caseous necrosis show large number of bacilli in their smears with predominant pattern of grade 3+. Whereas in smears with epithelioid granuloma alone the AFB bacilli were reduced in number with predominant grade being grade 1+. In smears with caseous necrosis and epithelioid granuloma the grading of AFB varies with the patients CD4 count. This was similar to the study as observed by Guru et al,<sup>12</sup> Gupta et al<sup>16</sup> and Das et al.<sup>17</sup>

The activity of the disease and the immune function of the patient can be assessed by the correlation of AFB grading with various patterns of tuberculosis as observed in FNAC.

The mean CD4 count decreased progressively with increase in the grading of AFB and appearance of necrosis. This was similar to the study as observed by Guru et al.<sup>12</sup>

## CONCLUSION

Lymphadenopathy is one of the clinical manifestations of HIV. Fine Needle Aspiration Cytology is a useful screening tool for evaluating the cause of lymphadenopathy. Thus, FNAC eliminates the need for surgical excision. It allows early treatment for specific conditions and there by reduces morbidity and mortality. Correlation of Fine Needle Aspiration Cytology finding with CD4 count and AFB staining helps the clinician to access the immune status of the affected individual. The mean CD4 count decreased progressively with increase in the grading of AFB and appearance of necrosis.

## REFERENCES

- Shobhana et al; People living with HIV infection/ AIDS- A study on Lymphnode FNAC and CD4 count. Indian journal of Medical Microbiology; 2002; 20; 2; 99-101
- Anthony S. Fauci, H. Clifford Lane: Harrison's Principles of Internal Medicine; Human Immunodeficiency Virus Disease: AIDS and related disorders. 18th edition; volume 1; Mc Graw Hill; 1506-1585.
- Tripathy S. Menon, Joshi DR. Preliminary observation on lymphocyte sub population in HIV positive and HIV negative tuberculous patients in Pune, India. Indian Journal of Med Res. 2000; 111; 195.
- Jones BE, Young SM, Antoniskis D: Relationship of manifestation of tuberculosis to CD4 count in patients with HIV infection. Am Rev Respir D; 1993, 148; 1292.
- Saikia UN, Dev p, Jindal B, Saikia B: Fine Needle Aspiration cytology in Lymphadenopathy of HIV- Positive cases. Acta cytological. 2001; 45; 589-592.
- Jayaram G, ChewMT; Fine Needle Aspiration Cytology of Lymphnode in HIV infected individuals. Acta cytological. 2000; 44; 960-966.
- Parikh U.R. et al 'Diagnostic utility of FNAC in HIV positive lymphadenopathy'- journal of clinical research letters, volume 3, issue 2, 2012, p-37-40.
- Martin Bates E., Tanner A et al Journal of clinical pathology, 1993, 46, 564-566.
- Vanisri et al "Fine Needle Aspiration Cytology findings in Human Deficiency Virus Lymphadenopathy" - Indian Journal of Pathology and Microbiology, 2008, volume 51, issue 4, pg 481-484.
- Shenoy R. Kapadi et al acta cytological, volume 46, 35-39.
- Sathyaranayana et al "Fine Needle Aspiration Cytology of lymph node in HIV infected patients" - Med J Armed Forces India 2002; 58: 33-37.
- Guru et al; "FNAC of Peripheral Lymphnodes in HIV- positive patients." Scientific medicine; 2009; 1.
- Ajay Jarya et al - "Manifestations of tuberculosis in HIV/AIDS patients and its relationship with CD4 count". Lung India. 2011 Oct-Dec; 28(4): 263-266.
- Grossi NA et al - "Utility of Fine Needle Aspiration in HIV patients with corresponding CD4 counts. Four years experience in a large inner city hospital. Acta cytol. 1997; 41:811-816.
- kumar s, et al: Acid fast staining patterns and their correlation with HIV positivity. Acta cytol. 2002; 46; 111-112.
- GuptaSK et al - "cytodiagnosis of tuberculous lymphadenitis: A correlative study with microbiological examination. Acta Cytol. 1993; 27; 329-333.

- Das DK et al - "tuberculous lymphadenitis: correlation of cellular components and necrosis in lymphnode aspirate with AFB positivity and bacillary count" Indian Journal of Pathology and Microbiology; 1990; 33; 1-10.