Fine Needle Aspiration Cytology and CD4 Count Estimation in HIV Positve Patients with Lymphadenopathy

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ABSTRACT

Introduction: Lymphadenopathy is among the earliest manifestation of many opportunistic infections and malignancy. Hence there is a need for simple investigations like FNAC for evaluation of HIV lymphadenopathy. This study was undertaken to analyze the cytological patterns of lymph node lesions in HIV/AIDS patients.

Material and Methods: In this study a total of 660 patients with lymphadenopathy were included of which 597 were HIV negative and 63 were HIV positive. Lymph node aspirates were stained with Giemsa and Ziehl Neilson stains. CD4 count were recorded. Tubercular lymphadenitis was further categorized. Acid-fast bacilli grading was done on Z-N positive smears. Each lesion was compared with CD4 count.

Results: There was increased prevalence of tubercular lymphadenitis in HIV positive patients. The most common opportunistic infection was tuberculosis. The maximum number of HIV negative patients were in the age range of 10-19 years, while 20-29 years of age group was the major group in HIV positive patients. Epithelioid cell granuloma with caseous necrosis was the commonest cytological picture in HIV positive patients, 37% show AFB positivity with 2/3 cases had grade IVAFB positivity. Maximum number of patients were found with CD4 count range of 201-300cells/µl.

Conclusion: Lymph node cytology was found to be a useful tool in lymphadenopathy cases for identification of oppertunistic infection, non neoplastic and neoplastic lesions. Comparison of these lesions with CD4 count and AFB grading reflects immunity and disease activity aiding better treatment.

Keywords: lymphadenopathy, CD4 count, HIV patients

INTRODUCTION

The Human immunodeficiency virus (HIV) infection leading to acquired Immuno Deficiency syndrome (AIDS) is considered to be one of the major public health problem. According to UNIAIDS report on the global AIDS epidemic (2012) 34 million people are living with HIV.[1]

AIDS was first recognized in US in 1981. In 1983, HIV virus was isolated from lymphnode and in 1984, this virus was demonstrated to be causative agent of AIDS. In India first AIDS case was reported in a transfusion recipient in 1986. There are 2.39 million people with AIDS/HIV in India.[2]

With the identification of HIV is 1983, as a causative agent of AIDS, the CDC classified HIV infected individuals on the basis of clinical conditions associated with HIV infection and CD4 count.

Using this system, any HIV infected individual with a CD4+ cell count <200 cells/ul has AIDS by definition regardless of presence of symptoms or opportunistic disease.

Many individuals with primary HIV injection many have generalized lymphademopathy. Lymphnode is most favoured site for initial infection during disease progression.[3,4] All the lymphnodes can be easily sampled by the needle aspiration,[5] Which can be further used for other ancillary studies.[6]

This study was performed with the aim to know the prevalence of different opportunistic infections in HIV/AIDS patients in comparison to general population, to study the frequency of TB in HIV/AIDS patients in comparison to other infection, to study CD4 count in HIV positive patients with lymphadenopathy and to find out correlation of different infections with CD4 count.

MATERIAL AND METHODS

The study was a prospective study conducted with study period from June 2014 to May 2015. A total of 660 patients with lymphadenopathy were included (based on inclusion and exclusion criteria), out of which 597 were of HIV negative and 63 patients were HIV positive, in the Department of Pathology, LLRM Medical College, Meerut. Prior to the study ethical clearance and informed consent were taken.

Inclusion criteria

1. Patients with lymphadenopathy of more than 1 cm size.
2. Patients of all age group and sex were included.

Exclusion criteria

1. Unwilling patients
2. Uncoperative patients

A detailed clinical history and clinical examination was conducted in all cases. Fine needle aspiration of lymph node with 22 bore needle and 20ml syringe was done to detect the cause of lymphadoropathy.

Following features were noted: -

1. *Type of aspirate* – Cheesy, Pus, Blood mixed, Nonspecific
2. Cytomosphological features on Giemsa stain
   a. Epithelioid cells / epitheloid call granuloma+ caseous necrosis
   b. Caseous necrosis only
   c. Acute and / or chronic inflammatory exudate + caseous necrosis
   d. Any combination of a+b+c
3. *Zn stain for AFB positivity* – (Ananthanarayan and Paniker's microbiology-7th edition)AFB reporting was done
4. Culture and sensitivity were performed wherever necessary.
5. *CD4 count estimation:* Diagnosis of HIV was done by ELISA test followed by CD4 counts by BD FACS count system.[7] Blood collected in K2 EDTA vacutainer tube. The BD FACS count system used for enumerating absolute lymphocyte counts (cells/µl whole blood) of CD3+T lymphocytes and CD4+T lymphocytes. % CD count is

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calculated by using the following formula. % CD4 count = $\frac{\text{Absolute CD4+T lymphocyte count}}{\text{Total Lymphocyte Count}}$

Absolute CD4+ T lymphocyte count: as obtained by the BD FACs count.

Total lymphocyte count can be obtained by a cell counter or alternatively obtained using the following formula.

Total lymphocyte count = $\frac{\text{Total number of lymphocytes (DLC)}}{100}$ X Total leucocyte count

STATISTICAL ANALYSIS

For data analysis, we used Statistical Package for the Social Sciences (SPSS) version 10. Age, gender, site of FNAC and cytomorphological patterns were expressed as frequency and percentage. Significance was estimated by chi square test.

RESULTS

The study was conducted with study period from June 2014 to May 2015 on 660 patients with lymphadenopathy, out of which 597 were of HIV negative and 63 patients were HIV positive, in the Department of Pathology, LLRM Medical College, Meerut.

Distribution of HIV positive / HIV negative patients with Lymphadenopathy according to age group

HIV negative patients were found in the age group of 1-89 years, whereas HIV positive patients were found in the age group of 1-69 years. For HIV negative group, maximum number of cases 165 (27.64%) were of 10-19 years and minimum number of patients 4 (0.67%) were of 80-89 years. For HIV positive group, maximum number of cases 26 (41.27%) were of 20-29 years and minimum number of patients 24 (38.10%) were of 30-39 years.

Distribution of HIV positive patients with lymphaderopathy according to site involved

In HIV positive group, most common site involved was cervical (42, 66.66%), followed by axillary(8, 12.70%), submandibular (5, 94%),post auricular (4, 6.35%),supraclavicular (3, 4.76%) and inguinal lymph nodes (1, 1.59%).

Distribution of HIV positive and negative patients with Lymphadenopathy according to diagnosis

Out of HIV positive patients, maximum cases (40/63) were of tubercular lymphadenitis followed by reactive hyperplasia (8/63), granulomatous lymphadenitis (8/63), pyogenic lymphadenitis (4/63) and lymphoma (1/63). Out of HIV negative patients, maximum cases (205/597) were of tubercular lymphadenitis followed by reactive hyperplasia (194/597), granulomatous lymphadenitis (75/597), lymphoma/malignancy (75/597) and pyogenic lymphadenitis (22/597).

Cytomorphological patternsof tubercular lymphadenitis in HIV patients

Out of 40 HIV positive patients with of tubercular lymphadenitis, most common Cytomorphological patterns observed was Epithelioid cell granuloma + caseousnecrosis (20,50%) followed by Epithelioid cell granuloma +caseous necrosis + acute/on chronic inflammation (10, 25%), Caseous necrosis + Acute/on chronic inflammation (7,17.5%), and Caseous necrosis (3,7.50%). Only 15 patients showed AFB positivity (Table-1).

Distribution of HIV positive patients with Lymphadenopathy according to sex and CD4 count

In this study, 48 male patients and 15 female patients. Maximum number of male patients (12) were seen in CD4 count range of 201-300/l and maximum number of female patients (9) were also seen in CD4 count range of 201-300/µl (Table-2)

CD4 count range in different Cytomorphological findings

Maximum number of TBLN (11), RHLN (5) Granulomatous (2) Pyogenic (2) were in CD4 count range of 201-300/l. One patient of NHL was in CD4 count range of <100/µl (Table-3).

Distribution of CD4 count in different Cytomorphological types of TBLN

In our study, total number of patients showing Epithelioid cell granuloma +Caseousnecrosis are 20 in which maximum number of 6 patients were inCD4 count range of <100/µl. total number of patients showing Epithelioid cell granuloma +Caseous necrosis and acute / on chronic inflammation are 10 in which maximum number of 4 patients were in CD4 count range of 201-300/µl and total number of patients showing Caseous necrosis and acute/on chronic inflammation are 7 in with maximum number

Cytomorphological features	No. of patients	Percentage
Epithelioid cell granuloma +caseous necrosis	20	50
Caseous necrosis + Acute / on chronic inflammation	7	17.5
Epithelioid cell granuloma +caseous necrosis + acute / on chronic inflammation	10	25
Caseous necrosis	3	7.50
AFB positivity	15	37.5

Table-1: Cytomorphological patternsof tubercular lymphadenitis in HIV patients

CD4 count range cells/µl	No. of patients	Male	Female	Percentage
<100	13	11	2	20.63
101-200	11	10	1	17.46
201-300	21	12	9	33.33
301-400	8	6	2	12.70
401-500	5	4	1	7.94
601-600	1	1	-	1.59
601-700	2	2	-	3.17
701-800	1	1	-	1.59
801-900	1	1	-	1.59
Total	63	48	15	100

Table-2: Distribution of HIV positive patients with Lymphadenopathy according to sex and CD4 count.

of 3 patients were inCD4 count range of 100-200/µl (Table-4).

Distribution of HIV positive patients according to mean CD4 count and disease

In our study, mean CD4 count in TBLN patients was 232.03/µl, whereas in RHLN mean CD4 count was 249.25/µl, and in pyogenic lymphadenitis mean CD4 count was 265.25/µl, in Granulomatous 376/µl One patient of NHL WAS with CD4 count 76/µl.2 cases show no opinion with CD4 count 165.5/µl.

DISCUSSION

Human immunodeficiency virus (HIV) belonging to subject of retroviruses called lentivirus is the causal agent of AIDS.

Lymphadenopathy is described in 75% of reported cases of HIV syndrome. In this study, 660 patients with Lymphadenopathy were taken, out of which 597 were HIV negative and 63 were HIV positive.

Out of HIV positive group, age group of 20-29 yrs. was most commonly involved (41.27%) followed by 30-39 years (38.14%) while in HIV negative group, age group of 10-19 years was most commonly involved (27.64%) followed by 20-29 years (23.95%). This study was similar to Vanisri et al[8] (44.4%), Neelima et al[9] (76% between 21-40 yrs) and Deshmukh et al[10] (36+45.4 = 81.7%) between 21-40 yrs.

According to site, most common site was cervical lymph node (66.66%) followed by axillary and Submandibular group of lymph nodes (12.7% and 7.94% respectively). This is similar to studies by others.[9,10]

Most common cytological diagnosis was tubercular lymphadenitis (40, 63-49%) followed by granulomatous (12.70%) and reactive lymphadenitis (12.7%) respectively in HIV positive patients.[8-11] Most common cytomorphological pattern and HIV positivity in our study was epithelioid cell granuloma + caseous necrosis (20,50%) and 15 (37.5%) which is similar to other studies (Deshmukh,[10] 40% Neelima[9] 51.85%, Vanisri 7.6% Satyanarayana.[12]

In our study of 63 HIV positive patients highest CD4 cont range was observed in pyogenic lymphadenitis (265.25/µl) followed by reactive (249.25 cases/µl), tubercular (232.02) and malignant (76/µl). While other studies show highest CD4 count in reactive lymphadenitis.[9,12-14]

Chi-square test was used to find out statistical significance of tubercular lymphadenitis in HIV positive patient in comparison to HIV negative patients. This indicated that increase in prevalence of tuberculosis in HIV positive patients with lymphadenitis in comparison to HIV negative patients is highly statistically significant (<0.001).

CONCLUSION

There was increased prevalence of tubercular lymphadenitis in HIV positive patients which was statistically significant. The most common opportunistic infection was tuberculosis. The maximum number of HIV negative patients were in the age range of 10-19 years, whle 20-29 years of age group was the major group in HIV positive patients. Epithelioid cell granuloma with caseous necrosis was the commonest cytological picture in HIV positive patients, 37% show AFB positivity with 2/3 cases had grade iv AFB positivity. Maximum number of patients were found with CD4 count range of 201-300cells/µl. 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

CD4 count (cells/µl)	Diseases						
	TBLN	RHLN	Gran LN	Pyo LN	NHL	No opinion	Total
<100	10	1	1	-	1	-	13
101-200	8	-	1	1	-	1	11
201-300	11	5	2	2	-	1	21
301-400	6	1	1	-	-	-	8
401-500	2	1	1	1	-	-	8
501-600	1	-	-	-	-	-	1
601-700	1	-	1	-	-	-	2
701-800	1	-	-	-	-	-	1
801-900	-	-	1	-	-	-	1
Total	40	8	8	4	1	-	63

TBLN: Tubercular lymphadenitis, RHLN: Reactivelymphadenitis, Gra. LN: Granulomatouslymphadenitis, Pyo. LN: Pyogenic lymphadenitis, NHL: Non Hodgkin's Lymphoma

Table-3: CD4 count range in different Cytomorphological findings

CD4 Count Cells/ µl	Cytomorphology				
	ECG+CN	ECG+CN +A/on ch. inf.	CN+A/on C. inf.	CN only	Total
<100	6	-	2	1	10
101-200	5	-	2	1	8
201-300	5	4	1	1	11
301-400	3	3	-	-	6
401-500	1	1	-	-	2
501-600	-	-	1	-	1
601-700	-	1	-	-	1
701-800	-	1	-	-	1
801-900	-	-	-	-	-
Total	20	10	7	3	40

ECG- Epithelioid cell granuloma CN= Caseous necrosis A/on C. inf. = Acute / on chronic inflammation

Table-4: Distribution of CD4 count in different Cytomorphological types of TBLN

in identifying majority of the reactive and neoplastic lesions and guides for the subsequent management of the patient.

REFERENCES

1. Global AIDS Response Progress Reporting 2012: guidelines – construction of core indicators for monitoring the 2011 political declaration on HIV/AIDS. Geneva: UNAIDS: 2012.
2. HIV Sentinel surveillance 2010-11: A technical brief. New Delhi: National AIDS control organization (Department of AIDS Control); 2012.
3. Bhoopat L, Patanasakpinyo C, Yanaranop M, Bhoopat T. Clinico-immunopathological alterations of lymph nodes from human immunodeficiency virus infected patients in northern Thailand. Asian Pac Allergy Immunol. 1999;17:85-92.
4. Lambertiuccl JR, Rayes AA, Nunes F, Landazuri Palacios JE, Norbe V. Fever of Undetermined origin in patients with the acquired immunodeficiency syndrome in Brazil: report on 55 cases. Reg. InstiMedi Trop S90 Paulo. 1999;41:27-32.
5. Gupta AK, Nayar M, Chandra M, Reliability and limitations of fine needle aspiration cytology of lymphadenopathies; an analysis of 1261 cases. Actacytol. 1991;35:777-83.
6. Prasad R, Garg SK, Mukerjee PK, Agarwal P.K. Role of fine needle aspiration cytology in the diagnosis of Lymphadenopathy, Indian J chest Dis Allied Sci. 1993;35:27-9.
7. BD FACS count system. Immuno cytometry system Users manual, BD. 2004,20-21.
8. Vanisri HR, Nandini NM, Sunila R, Fine Needle aspiration cytology findings in human immunodeficiency virus Lymphadenopathy. Indian Journal of Patho. Micro. 2008;51:481-4.
9. Neelima T, Prema Latha, Lymph nodes cytology n HIV seropositive cases with hematological alternations. Indian J med Res. 2014;139:301-307.
10. Deshmukh AT., Jagtap MW, Nomaan Nafees. Cytological evaluation of Lymphadenopathy in HIV patients Int. J. of Recent trends in Science and Technology. 6:125-129.
11. Shenoy R, Kapadi SN, Pai KP, Kini H, Mallya S, Khadilkar UN, et al, fine needle aspiration diagnosis in HIV related Lymphadenopathy in Mangalore, India, ActaCytol. 2002;45:35-9.
12. Satyanarayana S, Kalghati AT, Muralidhar A, Prasad RS. Jawed KZ, Trehan A. Fine needle aspiration cytology of lymph nodes in HIV infected patients. MJAF I. 2002;58:L33-37.
13. Shobhana A, Guha SK, Mitra K, Dasgupta A, Negi DK, Hazra SC. People living with HIV infection / AIDS: A study on lymphnode FNAC and CD4 count. Indian J med Microbiol. 2002;99-101.
14. Neha Singh, Abhishek Singh, Rashmi Chauhan, Preeti Singh, Nidhi Verma. Fine needle aspiration cytology in evaluation of lymphadenopathy in pediatric age group: our experience at tertiary care centre. International Journal of Contemporary Medical Research. 2016;3:1347-1351.

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