Original Research Paper

Pathology

STUDY OF LYMPH NODE CYTOLOGY IN HIV SEROPOSITIVE PATIENTS AND ITS CORRELATION WITH CD4 COUNT

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ABSTRACT HIV/AIDS is a major health problem globally. India bears the third highest HIV patients load worldwide. The aim of this study was to identify the different causes of lymphadenopathy among HIV infected patients in central India and correlate them with CD4 T cell counts. The clinical stage is useful for assessment at baseline or entry into long-term HIV care and in the follow-up of patients in care and treatment program. This study also aims to compare the cytomorphological diagnosis of lymph node with available clinical parameters and categorize the patient according to the WHO clinical staging for HIV and AIDS. In the present study 79 cases were evaluated. Fine Needle Aspiration Cytology (FNAC) was performed as an outpatient procedure in the Department of Pathology. Smears were stained routinely with Hematoxylin & Eosin, Papanicolaou stain, May-Grunwald Giemsa (MGG) and Ziehl Neelson stain (ZN) for acid fast bacilli (AFB). CD4 T cell count at the time of fine needle aspiration was used. Out of total 79 cases, 44 (55.70%) were typed as tubercular lymphadenitis, 14 (17.72%) as reactive lymphadenitis, 09 (11.40%) as suppurative lymphadenitis, 6 (7.59%) as granulomatous lymphadenitis, 3 (3.80%) as Non Hodgkin's lymphoma and 1 (1.26%) as metastases of squamous cell carcinoma. The WHO clinical staging was done for 77 cases. It included 7 (9.09%) cases in stage I, 7 (9.09%) cases in stage II, 16 (20.78%) cases in stage III and 47 (61.04%) cases in stage IV. CD4 T lymphocyte counts showed a descending pattern with progression of staging. Tubercular lymphadenitis constituted the most common condition in HIV positive lymph node aspirates. A cytological diagnosis of non-specific granulomatous lymphadenitis needs further etiological workup. FNAC findings in HIV lymphadenopathy are noticeably different in India in comparison to the western countries.

Acquired Immunodeficiency Syndrome (AIDS) is caused by a lymphotropic retrovirus of the genus Lentivirus, part of the family of Retroviridae.

There are two species of HIV known to exist: HIV-1 and HIV-2. HIV-1 is the virus that was initially discovered. It is more virulent, more infective, and is the cause of the majority of HIV infections globally. HIV-2 is largely confined to West Africa[1].

As the Human immunodeficiency virus primarily infects the lymphocytes, lymph nodes are involved in all stages of infection and lymphadenopathy is one of the earliest sign in HIV infected patients[2].

Many HIV seropositive patients can develop generalized lymphadeopathy that may resolve spontaneously or persist for months. If lymphadenopathy of size greater than 1 cm occurs in two or more extra inguinal sites and persists for more than three months with no other detectable cause on evaluation, the patient is diagnosed with persistent generalized lymphadenopathy (PGL). It is caused by follicular hyperplasia due to chronic HIV infection[3]. Apart from PGL, lymphadenopathy can also be a manifestation of opportunistic infection or a lymphoid malignancy.

HIV infection has basically three stages: acute infection, chronic asymptomatic phase and AIDS. Acute infection lasts an average of 28 days and can include symptoms such as fever, lymphadenopathy, pharyngitis, rash, myalgia, malaise, and mouth and esophageal sores. The chronic asymptomatic phase shows few or no symptoms and can last from two weeks to twenty years and beyond. AIDS, the final stage of HIV infection shows as symptoms of various opportunistic infections[3].

Histopathological analysis remains the gold standard for diagnosing lymphadenopathy, but it is time consuming and requires elaborate precautions. Fine needle aspiration cytology (FNAC) is a simple and rapid diagnostic technique. It gives early results and has thus become popular in a developing country like India with a large patient load.

World health organization (WHO) Clinical staging[4] is used once HIV infection has been confirmed (serological and/or virological evidence of HIV infection). The clinical stage is useful for assessment at baseline (first diagnosis of HIV infection) or entry into long-term HIV care and in the follow-up of patients in care and treatment program. It should be used to guide decisions on when to start co-trimoxazole prophylaxis and other HIV related interventions, including when to start antiretroviral therapy.

Objectives
1. To study the cytological spectrum of lymphadenopathy in HIV seropositive cases.
2. To correlate the cytomorphological diagnosis of lymphadenopathy with CD4 count.
3. To compare the cytomorphological diagnosis of lymph node with available clinical parameters and categorize the patient according to the WHO clinical staging for HIV and AIDS.

Material and Methods
The cross sectional study was undertaken in the Department of Pathology of our hospital over a period of 2 years. During this period 79 fine needle aspirates were obtained from 79 known HIV positive patients who presented with lymphadenopathy. All the patients were studied prospectively with informed consent of the patient.

All HIV positive patients with palpable lymph nodes > 0.5 cm in the smallest dimension and age greater than or equal to 15 years were included.

After taking written informed consent, FNAC was performed in the Cytology clinic or in the respective wards. All the smears were stained by Hematoxylin & Eosin, Papanicolaou stain, May-Grunwald Giemsa (MGG) and Ziehl Neelson stain (ZN stain) for acid fast bacilli (AFB). Special stains like Periodic Acid Schiff (PAS) and Gram's stain were done as and when necessary.

CD4 count of each patient was done with the help of flow

cytometer (The BD FACSCalibur™ system) by Becton Dickinson.

RESULTS

A total of 79 cases were studied. Maximum 31 (39.24%) patients belonged to age group of 31-40 years. The youngest patient was a 15 year old boy and the oldest patient was a 53 year old female. The mean age was 33.77 years. There were 47 (59.5%) males and 32 (40.5%). The male to female ratio was 1.47:1.

Table 1 – Clinical presentation of study cases

Sign/Symptoms	No. of patients	% of patients
Fever	33	41.77
Weight loss	33	41.77
Loss of appetite	31	39.24
Cough	13	16.45
Asymptomatic	4	5.06

The above table shows that the patients presented with multiple signs and symptoms, fever and weight loss being presented in 33 (41.77%) of patients.

Table 2 - Anatomic Sites of aspirates

Lymph nodes	Patients	% of Patients
Cervical	55	69.62
Axillary	12	69.62
Inguinal	05	6.33
Multiple	07	8.86
Total	79	100

Out of total 79 aspirates, 55 (69.62%) were from cervical group of lymph nodes, 12 (15.19%) were from axillary lymph nodes, 05 (6.33%) were from inguinal lymph nodes and 07 (8.86%) were from multiple (>2) regional lymph nodes.

Out of total 79 aspirates, 77 (97.46%) were adequate while 2 (2.54%) were inadequate for cytological interpretation.

Table 3 – Cytomorphological diagnosis

Cytomorphological diagnosis	No. of Patients	% of Patients
Tubercular lymphadenitis	44	55.70
Reactive lymphadenitis	14	17.72
Suppurative lymphadenitis	09	11.40
Granulomatous lymphadenitis	06	7.59
Non Hodgkin's lymphoma	03	3.80
Non diagnostic	02	2.53
Metastases	01	1.26
Total	79	100

Out of total 79 cases, 44 (55.70%) were typed as tubercular lymphadenitis, 14 (17.72%) as reactive lymphadenitis, 09 (11.40%) as suppurative lymphadenitis (figure – 1), 6 (7.59%) as granulomatous lymphadenitis, 3 (3.80%) as Non Hodgkin's lymphoma and 1 (1.26%) as metastases of squamous cell carcinoma. Gram stain was done in all the 9 cases of suppurative lymphadenitis, of which 3 showed presence of gram positive cocci (Figure- 1). Tubercular lymphadenitis constituted the most common condition comprising of 44 (55.70%) cases of all lymph node aspirates. There were 3 cytomorphological patterns of tubercular lymphadenitis as shown in the table below. All the 44 smears showed AFB positivity on Ziehl Neelsen stain

Table 4 - Tubercular lymphadenitis smear pattern (AFB positive)

Tubercular lymphadenitis Pattern	No. of cases	% of Patients
Granulomatous	3	6.81
Epithelioid granuloma with necrosis	22	50
Predominantly necrotic	19	43.19
Total	44	100

Epithelioid granuloma with necrosis was the most common pattern observed in 22 (50%) cases. Smears showed clusters of loosely cohesive epithelioid histiocytes in a necrotic background (Figure- 2). A predominantly necrotic pattern was the second most common pattern observed in 19 (43.19%) cases. The smear showed caseous material or thin necrotic debris. Granulomatous pattern was observed in 3 (6.81%) cases. Smears showed only epithelioid cell granulomas without necrosis. We found 3 (3.80%) cases of Non Hodgkin's Lymphoma. One case diagnosed as DLBCL (figure – 3) and one case labeled as Plasmablastic lymphoma (figure - 4). The remainder could not be sub typed further.

Table 5 - Comparison of Cytomorphological diagnosis and CD4 T lymphocyte counts

Cytomorphological diagnosis	No. of Patients (n = 77)	% of Patients	Mean CD4 Count (50-550/cumm)
Tubercular lymphadenitis	44	55.70	186
Reactive lymphadenitis	14	17.72	403.78
Suppurative lymphadenitis	09	11.40	227.77
Granulomatous lymphadenitis	06	7.59	359
Non-Hodgkin's lymphoma	03	3.80	113.33
Metastases	01	1.26	398
Total	77	100	

Absolute CD4 T lymphocyte counts were available in 77 of the total 79 cases. It varied from 50 to 550 cells / μl (mean CD4 T lymphocyte count - 244.59/μl). The above table shows highest mean CD4 T lymphocyte count (403.78/μl) in reactive lymphadenitis and lowest (113.33/μl) in Non Hodgkin's lymphoma.

Table 6 – Correlation of TB lymphadenitis Pattern with CD4 Count (n=44)

Tubercular Lymphadenitis Pattern	No. of Patients	% of Patients	CD4 Count (cells/μl)
Granulomatous	3	6.81	435
Epithelioid granuloma with necrosis	22	50	236.90
Predominantly necrotic	19	43.19	117.52
Total	44	100	

Absolute CD4 T lymphocyte count, in the 44 patients of tubercular lymphadenitis varied from 50 to 550/μl. The mean count was 186/μl. The mean count decreased with increasing necrosis.. A total of 4 cases of tubercular lymphadenitis had CD4 counts $\geq 350/\mu l$ and 40 cases had counts $\leq 350/\mu l$. A chi square test was applied and we found that the correlation was statistically significant. ($x^2 = 11.53$, p = 0.0006).

Total 14 (17.72%) patients had reactive lymphadenitis. In these patients absolute CD4 T lymphocyte count ranged from 198 to 456 /μl. The mean CD4 T lymphocyte was 403.78 /μl. Out of total 14 cases of reactive lymphadenitis, 10 cases had CD4 T lymphocyte counts ≥ 350 cells/μl and 4 had CD4 T lymphocyte counts $\leq 350/\mu l$. A chi square test was applied and we found that the correlation was statistically significant ($x^2 = 17.17$, p = 0.00003).

Total 09 (11.40%) patients were labeled as suppurative lymphadenitis. Absolute CD4 T lymphocyte count ranged from 71 to 341/μl. The mean CD4 T lymphocyte count for these patients was 227.77/μl. All 9 cases had CD4 T lymphocyte count $\leq 350/\mu l$. A chi square test was applied and we found that the correlation was statistically significant ($x^2 = 3.33$, p = 0.03).

Total 06 (7.59%) patients were labeled as non specific granulomatous lymphadenitis. In these patients absolute CD4 count ranged from 105 to 609/μl. The mean CD4 count

was 359/μl.Out of total 6 cases of granulomatous lymphadenitis, 4 cases had CD4 T lymphocyte counts ≥ 350/μl and 2 cases had counts ≤ 350/μl. A chi square test was applied and we found that the correlation was statistically significant. (x^2 = 3.966, p = 0.04)

Table 7 - Comparison of Clinical and Cyto-diagnosis with CD4 Count (n=77)

WHO Clinical Staging No. of cases (%)	Cytological Diagnoses No. of cases (%)	Mean CD4Count (cells / μl)
Stage I 7 (9.09%)	Reactive lymphadenitis – 6 (7.8%) Metastases – 1(1.29%)	465.85
Stage II 7 (9.09%)	Reactive lymphadenitis – 3 (3.9%) Suppurative lymphadenitis – 2 (2.6%) Granulomatous lymphadenitis – 2 (2.6%)	379.28
Stage III 16 (20.78%)	Reactive lymphadenitis – 5 (6.5%) Suppurative lymphadenitis – 7 (9.09%) Granulomatous lymphadenitis - 4 (5.19%)	267.18
Stage IV 47 (61.04%)	Tubercular lymphadenitis – 44 (57.14%) NHL – 3 (3.89%)	183.89

The WHO clinical staging was done for 77 cases. It included 7 (9.09%) cases in stage I, 7 (9.09%) cases in stage II, 16 (20.78%) cases in stage III and 47 (61.04%) cases in stage IV. CD4 T lymphocyte counts showed a descending pattern with progression of staging.
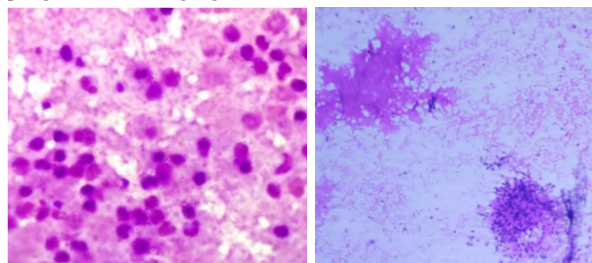
Figure 1 – Suppurative Lymphadenitis showing Gram Positive cocci along with neutrophils (Gram's stain)

Figure 2 – Epithelioid cell granuloma in a background of necrosis
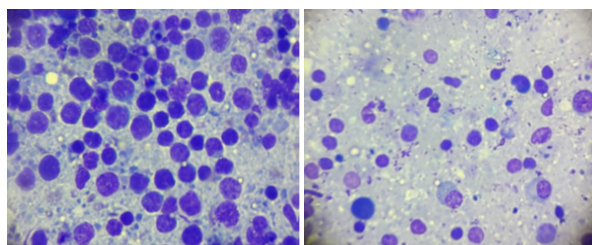
Figure 3 – Diffuse Large B cell lymphoma showing a large population of mainly centroblasts

Figure 4 – Smear shows a mixture of cells having large nuclei along with plasmacytic cells

DISCUSSION

In our study we found that majority of the patients presented with fever (41.77%) and weight loss (41.77%). Tirumalasetti et al (2014)5 and Mondal et al (2015)6 also found fever to be the commonest symptom (84.49%).

Table 8 - Comparison of cytomorphological diagnosis of different authors

Author (Year)	Sample size	Tubercular %	Reactive %	Suppurative %	Granulomatous %	NHL %	Fungal %	Metastases %
Reid et al (1998)[7]	52	15.38	38	12.5	--	9.23	1.53	--
Shenoy et al (2002)[8]	48	48	36	2	--	10	-	2
Satyanarayana et al (2002)[9]	196	42.3	34.2	---	--	2	1	--
Vanishri et al (2008)[10]	36	36.1	58.3	2.7	--	2.7	--	--
Lowe et al (2008)[11]	73	11	34.3	4.1	2.7	5.47	--	--
Deshmukh et al (2013)[12]	44	45.4	29.5	6.81	18.1	--	--	--
Tirumalasetti et al (2014)[5]	129	41.9	35.6	12.4	0.77	3.1	0.77	0.77
Khiste et al (2015)[13]	58	55.17	17.24	27.58	--	3.44	--	--
Nasser et al (2017)[14]	110	53.6	27.1	6.4		2.7	0.9	1.9
Present study (2017)	79	55.70	17.72	11.4	7.59	3.8	--	1.26

The above table shows a comparison of cytomorphological diagnosis of present study with the studies of other authors.

Among the studies conducted in the Indian subcontinent, majority showed that Tubercular lymphadenitis was the most common diagnosis as observed in studies conducted by Shenoy et al (2002)[8], Tirumalasetti et al(2014)[5], Khiste et al(2015)[13] and Nasser et al (2017)[14]. Our findings were in agreement to the above studies. However the studies conducted by the two foreign authors, Reid et al (1998)[7] and Lowe et al (2008)[11] showed reactive lymphadenitis as the most common diagnosis. This observation points to the increased prevalence of tuberculosis in the Indian subcontinent[15].

We chose AFB positivity to be the sole criterion for labeling a diagnosis as tubercular lymphadenitis. A similar methodology was adopted by Satyanarayana et al (2002)[9], Deshmukh et al (2013)[12] and Kumar et al (2016)[16].

We found three cyto-morphological patterns of tubercular lymphadenitis. They are granulomatous, epithelioid granuloma with necrosis and predominantly necrotic. These three patterns are similar to those found by Tirumalasetti et al (2014)[5], Khiste et al (2015)[13] as well as those given in the textbook "Diagnostic Cytopathology"[17].

We found lymph nodes showing epithelioid granuloma with necrosis to be the commonest 22 (50%) cases, followed by lymph nodes showing predominantly necrotic pattern 19 (43.19%) and well formed granulomas 3 (6.81%). Similar findings were noted by Shenoy et al (2002)[8], Deshmukh et al (2013)[12], Tirumalasetti et al (2014)[5] and Mondal et al (2015)[6]. The mean CD4 T lymphocyte count decreased with increasing necrosis in the smears. A similar trend was noted in the studies by Tirumalasetti et al (2014)[5], Mondal et al (2015)[6] and Kumar et al (2016)[16].

Reactive lymphadenitis was the second most common cytomorphological entity in our study comprising of 14 (17.72%) cases. The changes of HIV lymphadenopathy/ persistent generalized lymphadenopathy however could not be differentiated from reactive lymphadenitis with different etiology in the present study. In these patients, the mean CD4 count was 403.78/μl. This finding was consistent with the findings of Deshmukh et al (2013)[12], Ratan et al (2014)[18], Khiste et al (2015)[13] and Nasser et al (2017)[14] where the mean CD4 counts were 471.75/μl, 328/μl, 493/μl and 434.4/μl respectively. We found that the correlation was statistically significant in the present study. Nasser et al (2017)[14], however found no significant correlation between reactive lymphadenitis and CD4 counts (x^2 = 2.6, p > 0.05).

In the present study 09 (11.40%) cases were labeled as suppurative lymphadenitis. Similar findings were reported by Tirumalasetti et al (2014)[5] who found 16 (12.4%) cases of suppurative lymphadenitis. In the 9 (11.40%) patients of suppurative lymphadenitis, the mean CD4 count for these patients was 227.77/μl. Our findings were in agreement with the findings of Tirumalasetti et al (2014)[5], Ratan et al (2014)[18] and Nasser et al (2017)[14] who found the mean CD4 count associated with suppurative lymphadenitis to be 181.65/μl, 189.71/μl and 181.4/μl respectively. We found this correlation to be statistically significant. (x^2 = 3.33, p = 0.03). A similar finding was noted by Nasser et al (2017)[14] (x^2 = 8.87, p < 0.05).

In the present study 6 (7.59%) cases were labeled as granulomatous lymphadenitis. All cases were found to be negative for ZN stain. No further investigations (culture, PCR etc.) were done in these cases to rule out tuberculosis. Ratan et al (2014)[18] reported similar findings, where non-specific granulomatous lymphadenitis comprised of 7 (9.9%) cases. The mean CD4 count for the patients in our study was 359 cells/μl. Our findings were consistent with the findings of Deshmukh et al (2013)[12] who found the mean CD4 count to be 316.66/μl. Ratan et al (2014)[18] however found this value to be much lower (155.86/μl).

The WHO clinical staging showed 7 (9.09%) cases in stage I, 7 (9.09%) cases in stage II, 16 (20.78%) cases in stage III and 47 (61.04%) cases in stage IV. CD4 counts showed a descending pattern with progression of staging. Tirumalasetti et al (2014)[5] observed a mean CD4 count of 331.16 cells/μl, 235.5 cells/μl, 127.8 cells/μl and 79.91 cells/μl for patients in stage I, stage II, stage III and stage IV respectively. Nasser et al (2017)[14] also observed similar findings.

CONCLUSION

FNAC is a primary, easy and effective diagnostic modality for HIV lymphadenopathy patients which can identify majority of the reactive and neoplastic lesions and opportunistic infections and further guide the subsequent management of the patient. Tubercular lymphadenitis constituted the most common condition in HIV positive lymph node aspirates. A cytological diagnosis of non-specific granulomatous lymphadenitis needs further etiological workup. Correlation of lesion with CD4 T lymphocyte count provides information about the immune status and stage of the disease. FNAC findings in HIV lymphadenopathy are noticeably different in India in comparison to the western countries.

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