Original Research Article

Spectrum of morphological changes in lymph nodes of HIV infected patients with lymphadenopathy

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A B S T R A C T

Introduction: Lymphadenopathy is frequent in persons with HIV infection, occurring either as one of the earliest manifestations of infection or as a finding at any time during the course of disease. The evaluation of lymph node morphology has been one approach to diagnosis and in understanding the nature of the immune dysfunction in these conditions.

Aim: To identify the various causes of lymphadenitis and evaluate the various morphologic patterns in HIV positive patients with lymphadenopathy.

Materials and Methods: Total eighty lymph node biopsies were examined. Six lymph nodes were found to be inadequate. The precise history of the patients which included ESR, fever, opportunistic infection, any other complaints, CD4+, CD8+ counts were noted. The different histological changes in the lymph nodes were evaluated. Special stain was used to detect the microorganisms.

Results: Reactive hyperplasia was the most common cause of lymphadenitis. Type B pattern was seen in most of the patients. The type of pattern correlated with the CD4 counts. Tuberculosis was the most common opportunistic infection.

Conclusion: Lymph nodes biopsy is a valuable tool in the evaluation of HIV positive patients to identify the cause of lymphadenopathy.

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1. Introduction

Persistent generalized lymphadenopathy (PGL) is one of the earliest recognized clinical symptoms and present in one third of the infected patients of HIV.¹ It was defined as lymphadenopathy of at least 3 months duration involving two or more noncontiguous extra inguinal sites.² Persistent generalized lymphadenopathy occurs in the absence of opportunistic infections and is known as HIV-related lymphadenopathy. In HIV patients the common conditions affecting lymph node are: Reactive changes, Opportunistic infection and Neoplasm.

The evaluation of lymph node morphology has been one approach to diagnosis and in understanding the nature of the immune dysfunction in these conditions.

2. Aims

The present study has been undertaken in Human Immunodeficiency Virus (HIV) positive patients to:

1. Evaluate the common presenting features.
2. Correlate the CD4+ counts with the progression of HIV infection and disease outcome, wherever feasible.
3. Emphasize the usefulness of lymph node biopsy in diagnosis, prognosis.
4. Study the histological features of various lesions in the lymph nodes.
3. Materials and Methods

This study was conducted in department of Pathology, Government Medical College, Kota, over a period of three years, from January 2013 to December 2016.

Total eighty lymph node biopsies were examined. Six lymph nodes were found to be inadequate. The precise history of the patients which included ESR, fever, opportunistic infection, any other complaints, CD4+, CD8+ counts were noted. The different histological changes in the lymph nodes were evaluated. Special stain was used to detect the microorganisms.

Patients aged less than 14 years and inadequate samples were excluded from the study. The H & E stained sections of 10% formalin fixed lymph nodes tissue were studied. AFB stain is done by using Ziehl-Neelson method if H & E stained slides showed features suggestive of tuberculosis. The histologic findings of HIV positive reactive lymphadenopathy patients are compared with same in immunocompetent patients and the differences are noted.

4. Results

In the present study, reactive hyperplasia was the most common cause of lymphadenitis followed by tuberculosis. The peak age was found to be 25-45yrs with male predominance. The common complaints were pyrexia, weight loss, cough and generalized lymphadenopathy. Cervical lymph nodes were more commonly involved than other groups of nodes. Hepato-splenomegaly was found in 40% of patient. The ESR ranged from 40-140mm/hr and the CD4+ counts varied from 15-618/μl. Total 36 lymph nodes showed features of reactive hyperplasia. The various morphological features of reactive nodes were evaluated [Table 1].

Based on the above morphologic features, the lymph node pattern was categorized into three groups namely pattern A, B, C. (Table 2). Type A pattern has hyperplastic irregular follicles with monocytoid B cell hyperplasia. Type B pattern show effacement of follicles, involution of germinal centre and accumulation of hyaline material. There is marked proliferation of endothelial cells, depletion of lymphocytes and accumulation of plasma cells in paracortex. Type C pattern has atrophic or burnt out follicles with fibrosis and hyalinised germinal centres. The vessels are thick.

In the current study, type B pattern was observed in 28 cases (77.8%) whereas type A was seen in 8 cases (22.2%). No cases with Type C pattern were noted.

The histological features of type A and type B pattern were compared (Table 3).

The large follicles and sinus histiocytes were more commonly seen in type A pattern whereas follicle lysis was seen in 92.8% cases of type B pattern.

CD4 counts were available in 32 cases (88.9%) of reactive lymphadenitis. The mean CD4 cell count of 390/μl was seen in type A HIV lymphadenopathy while it was 155,50/μl in patients with type B pattern (Table 4).

The various morphological features of reactive hyperplasia in HIV positive patients were compared with non HIV controls (Table 5). For the latter group, 12 cases of lymph nodes with non specific reactive follicular hyperplasia were obtained. The various clinical settings in which these lymph nodes were evaluated are breast carcinoma, colonic carcinoma, choledocholithiasis and non specific reactive hyperplasia.

Tuberculosis was found to be the most common opportunistic infection. The various morphological features and its correlation with CD4 counts and AFB were evaluated (Tables 6, 7 and 8).

The various morphological features analyzed in cases of tuberculosis included; presence and type of granulomas, neutrophilic microabscess, periadenitis, plasmacytosis and paracortical expansion. In the present study, granulomas were detected in 90.9% of the cases and were the most common and conspicuous feature. Among the 30 cases with granulomas, caseous necrosis was found in 28 (93.3%). Confluent granulomas were more commonly seen than discrete ones. Neutrophilic microabscess with granular debris without coexisting granulomas was found in 11 cases. The bacillary load was found to be higher in this group.

Two cases of Histoplasmosis, Non Hodgkin Lymphoma and atypical mycobacteria were also diagnosed.

5. Discussion

Lymph node biopsies are frequently done in HIV positive patients. In the course of AIDS, a lymph node biopsy may elicit the cause of the lymphadenopathy or provide the etiologic diagnosis of a systemic disease. Tournieu et al have remarked on the importance attached to lymph node biopsy evaluation in HIV individuals in his article. He suggested that the light microscopic features with detail examination are very suggestive of AIDS. 8

Harry et al explored the histologic features of lymph node biopsy of 79 HIV positive patients and found three histological patterns. 9

1. Follicular hyperplasia with cytolysis.
2. Follicular involution with hyper vascularity.
3. Combination of the 1 and 2.

Patients with follicular hyperplasia with cytolysis were noted to have better prognosis than other two. So these workers opined that the all histological patterns represent stages of progression in HIV infection. Similar observations were also made by H J C Rashleigh Belcher et al. 10

The most common histopathological changes seen are mainly due to the B-region reactivity. This is expressed by
Fig. 1: Reactive lymphadenitis. a): Follicle with mantle zone hyperplasia (H&E 10X); b): Follicle lysis (H&E 10X), c): showing hyalinisation within arterioles of germinal center (H&E 20X); d): Monocytoid cells (H&E 20X)

Fig. 2: Tuberculosis lymphadenitis; a): Neutrophilic microabscess with granular debris (H&E 10X); b): Granulomas (H&E 10X); c): Caseous necrosis (H&E 10X); d): Histiocytes filled with histoplasma (H&E 40X)
Table 1: The various histopathologic features evaluated in these cases are shown Morphologic features (n=36)

| Lymph node compartment | Morphological features                  | No. of cases (%) |
|------------------------|----------------------------------------|------------------|
|                        | Size of follicles (Large)              | 13 (36.1)        |
| Germinal center        | Follicle lysis                         | 27 (75)          |
|                        | Mantle zone effacement                 | 14 (38.9)        |
|                        | Hemorrhage                             | 4 (11.1)         |
|                        | Endothelial cells                      | 36 (100)         |
| Para cortex            | Para cortex expansion                  | 17 (47.2)        |
|                        | Dermatopathic features                 | 16 (44)          |
|                        | Monocytoid cells                       | 13 (36.1)        |
| Sinuses                | Sinus histiocytosis                    | 3 (8.3)          |
| Others                 | Plasmacytosis                          | 13 (36.1)        |

Table 2: HIV lymphadenopathy- Type of pattern. (n=36)

| Pattern | Number of cases | Percentage % |
|---------|-----------------|--------------|
| Type A  | 8               | 22.2         |
| Type B  | 28              | 77.8         |
| Type C  | 0               | 0            |

Table 3: Morphological features in Type A and Type B pattern

| Lymph node compartment | Morphological features                  | Type A (n=8) | Type B (n=28) |
|------------------------|----------------------------------------|--------------|---------------|
|                        | Size of the follicles (Large)          | 6, 75%       | 7, 25%        |
| Germinal center        | Follicle lysis                         | 0, 0%        | 26, 92.8%     |
|                        | Mantle zone effacement                 | 0, 0%        | 14, 50%       |
|                        | Hemorrhage                             | 0, 0%        | 4, 14.3%      |
|                        | Endothelial cells (plump)              | 8, 100%      | 28, 100%      |
| Paracortex             | Para cortex expansion                  | 3, 37.5%     | 14, 50%       |
|                        | Dermatopathic features                 | 4, 50%       | 12, 42.9%     |
|                        | Monocytoid cells                       | 5, 62.5%     | 8, 28.6%      |
| Sinuses                | Sinus histiocytosis                    | 2, 25%       | 1, 3.6%       |
| Others                 | Plasmacytosis                          | 4, 50%       | 9, 32.1%      |

Table 4: HIV lymphadenopathy: Correlation between pattern and CD4+ count

| Pattern | CD4+ counts/μl |
|---------|-----------------|
| A       | 390             |
| C       | 155.5           |
|         | 0               |

Table 5: Comparison of morphological features of reactive lymph nodes in HIV and non HIV patients

| Lymph node compartment | Morphological features | HIV +ve patients (n=36) | HIV –ve patients (n=12) |
|------------------------|------------------------|------------------------|------------------------|
|                        | No. of patients | %                      | No. of patients | %                      |
| Germinal centre        | Small            | 8                     | 22.2                 | 8                     | 66.7                 |
|                        | Size of follicle  | Medium                | 13                   | 36.1                  | 4                    | 33.3                  |
|                        |                | Large                  | 13                   | 36.1                  | 0                    | 0                     |
|                        | Follicle lysis   | 27                    | 75                   | 3                     | 25                   |
|                        | Mantle zone effacement | 14 | 38.9 | 1 | 8.3 |
|                        | Hemorrhage       | 4                     | 11.1                 | 1                     | 8.3                  |
|                        | Endothelial cells| 36                    | 100                  | 12                    | 100                  |
| Paracortex             | Para cortex expansion | 17 | 47.2 | 10 | 83.3 |
|                        | Dermatopathic features | 16 | 44  | 7  | 58.3  |
|                        | Monocytoid cells  | 13                    | 36.1                 | 4                     | 33.3                 |
| Sinuses                | Sinus histiocytosis | 3                    | 8.3                  | 6                     | 50                   |
| Others                 | Medullary plasmacytosis | 13 | 36.1 | 10 | 83.3 |
Table 6: Tuberculosis: Microscopic features (n=33)

| Features                 | Number of cases | Percentage |
|--------------------------|-----------------|------------|
| Granulomas               | 30              | 90.9       |
| Neutrophilic micro-abscess| 11              | 33.3       |
| Periadenitis             | 10              | 30.3       |
| Plasmacytosis            | 19              | 57.6       |
| Paracortical expansion   | 04              | 12.1       |
| AFB positivity           | 33              | 100        |

Table 7: Type of granulomas (n=30)

| Granulomas         | Discrete | Confluent | Both |
|--------------------|----------|-----------|------|
| Caseating(n=28)    | 9(32.1%) | 14(50%)   | 5(17.9%) |
| Non-caseating(n=2) | 0        | 2(100%)   | 0    |

Table 8: Correlation between micro abscess formation, CD4+ count and AFB (n=11)

| CD4+ in μl | Microabscess | AFB <1/100HPF | AFB 1-10/HPF | AFB >10/HPF |
|------------|--------------|---------------|--------------|-------------|
| <40        | 6            | Nil           | 3            | 3           |
| 120-160    | 2            | Nil           | 1            | 1           |
| >200       | 3            | 2             | 1            | Nil         |

Table 9: The histological alteration seen in HIV related lymphadenopathy has been described in different terms by many authors

| Current | Jaffe et al | Iaochim et al | Pilieri et al | Schurrman et al | Burns et al |
|---------|-------------|---------------|---------------|-----------------|-------------|
| Florid follicular hyperplasia | Reactive follicular | Type I | Follicular hyperplasia | Follicular | Reactive follicular |
| Mixed | follicular | Type II | Mixed follicular and interfollicular hyperplasia | hyperplasia stage | hyperplasia |
| Follicular involution | Lymphocytic depletion | Type III | Lymphocytic depleting | Lymphocytic depletion | Lymphocytic depletion |
| Lymphocytic depletion | Burned out | | | | |

florid follicular hyperplasia and serves as reservoir of virus. This change is followed by depletion of CD4+ positive cells later. So the histological pattern shows a spectrum of changes ranging from marked lymphoid hyperplasia to marked lymphocytic depletion.11

In the current study, type B pattern was observed in 28 cases (77.8%) whereas type A was seen in 8 cases (22.2%). No cases with Type C pattern were noted. Ioachim et al had described the patterns similar to the current study.4 Amy et al had divided HIV lymphadenopathy into four groups i.e explosive follicular hyperplasia, mixed, follicular involution and lymphocytic depletion.1 Lymph nodes with type A pattern showed enlarged coalescing lymphoid follicles taking serpiginous and hourglass shapes. The germinal center were often irregular and hyperplastic, with numerous apoptotic bodies and tingible body macrophages. Minor follicle lysis was present. However, a few follicles showed severe follicle lysis. The mantle zone was intact in most of the follicles and a few had thick mantle zone. There were multiple focal aggregates of monocytoid cells located along the sub capsular sinuses, fibrous septa. One case showed castelman like features. Similar findings were observed by Paiva,12 Ioachim,4 Carlo,13 Amy1 and coworkers in their studies. In type B pattern, the germinal centers were disrupted by severe follicle lysis along with mantle zone effacement. However, few well formed follicles, hyalinization within germinal center and a pronounced increase of plasma cells were also noted.

Follicle lysis superficially resembles progressive transformation of germinal centers. However, in progressive transformation the influx of mantle zone cells seems to involve the entire circumference symmetrically.

Type B pattern may represent an adverse progression from that of type A pattern and appears to represent a transition from PGL to AIDS. It was named as Type II pattern by Edwin et al14 and follicular involution by Carlo et al13 and Paiva et al.12 Carlo et al has noted that hyper vascular follicular pattern with small follicles like Castelman’s disease are more commonly seen in this pattern.13

In the present study, type B was the most common pattern encountered. All the patients with type B pattern had numerous opportunistic infections. They were diagnosed as AIDS. However, two cases with type A also had opportunistic infection. Amy et al had observed in their study that progressive lymph node histology correlates
with the clinical course of patients.\textsuperscript{1} Hence lymph node biopsy and histologic examination can be used as an additional procedure for the treatment and care of AIDS patients. Maintenance of germinal centers depends on T-helper cells, and the disappearance of germinal centers from a hyperplastic lymph node is a morphologic clue to a significant loss of these cells.\textsuperscript{15}

The CD4 T lymphocyte count progressively decreases with progression of disease is represented by patterns of reactive change from type A to type B. These findings are similar to those of various workers (Edwin et al,\textsuperscript{14} Amy et al,\textsuperscript{1} Paiva et al.\textsuperscript{12}).

The various microscopic features of HIV lymphadenopathy are however may not be specific. To evaluate this, in the present study all morphological features were compared with 12 known non HIV cases. It was found that small sized well formed lymphoid follicles were more commonly seen in non specific reactive hyperplasia and follicle lysis was absent. HIV lymphadenopathy showed enlarged coalescing lymphoid follicles taking serpiginous and hourglass shapes. Most of the cases showed follicle lysis. Rest of the features like dermatopathic, paracortex expansion, monocytoid B cell, and hemorrhage within germinal cells were seen in both the groups.

Tuberculosis was the second most important cause of lymphadenopathy in the current study. In studies conducted by ‘Chaissen et al\textsuperscript{16} and Duncanson\textsuperscript{17} and coworkers, it was noted that TB usually precedes the diagnosis of AIDS, presumably because TB is more virulent than other HIV associated pathogens. Their data also confirms that TB is an AIDS related opportunistic infection characterized by atypical clinical and radiological features and poor survival.

Gnana et al\textsuperscript{18} noted a greater prevalence of extrapulmonary and disseminated forms of TB in HIV positive individuals than in immunocompetent patients. It is important to recognize this as it is an AIDS defining illness. The most frequent forms are lymphadenitis and miliary disease.

Lymph nodes are commonly infected by mycobacterium in patients with AIDS. In such patients, abscesses and purulent inflammation comprise a significant component of the histologic features of tuberculous lymphadenitis. Morphologically, in addition to the presence of polymorphs, the other criterias for the diagnosis are caseous necrosis and granulomas, of which the former is the most specific and sensitive as shown by Arora et al,\textsuperscript{19} Nambuya et al,\textsuperscript{20} Wannakrairrot et al.\textsuperscript{21} It is seen that granulomas tend to be less numerous in AIDS than in non AIDS patients.\textsuperscript{21} The various studies show that granulomas with epithelioid cells though not a definitive diagnostic feature, are indicative of tuberculosis unless proved otherwise. The presence of necrosis should always trigger special studies for mycobacteria. The yield of stainable AFB with necrotic debris is much higher in the later cases than in patients with granulomatous inflammation. If non caseous granuloma are found one should also rule out opportunistic fungal infections, of which Candida, Cryptococcus neoformsans, Histoplasma capsulatum have been reported in HIV positive patients. Hence, in cases with non casening granulomas, AFB stain is very useful to identify tubercle bacilli and thereby ascertain the etiology of the granulomatous process. Hence the gold standard for a definitive diagnosis of tuberculosis rests on the demonstration of AFB on histopathology or by culture studies.

Atypical mycobacterial infections are also seen with an increased frequency in patients with AIDS, the most common being the Mycobacterium Avium Intracellulare complex. Two cases were diagnosed in the present study. Micro abscess are more commonly seen in this though it is not specific. Kraus et al\textsuperscript{22} had opined that culture should be done in presence of microabscess and ill defined granulomas to rule out atypical mycobacteria.

Histoplasmosis is a systemic infectious disease that appears to be the second most life threatening mycosis in patients with AIDS. The predominant pathologic features in the affected organ are marked histiocytic proliferation. In the current study two cases of histoplasmosis were diagnosed and both the patients had CD4+ counts less than 30/\mu L. The major differential diagnosis on light microscopy includes Cryptococcus Neoformsans, Leishmania, and Toxoplasmosis. Mucicarmine and PAS clearly stain the capsule of Cryptococcus neoformsans whereas Histoplasma remain unstained. Toxoplasmosis is smaller and is not intracellular. Leishmania do not stain with silver. Bates et al,\textsuperscript{23} Jayaram and co-workers\textsuperscript{24} have also documented lymph nodes histoplasmosis in their studies.

Non Hodgkin lymphoma is a common neoplasm in AIDS patients. Ioachim,\textsuperscript{4} Jayaram\textsuperscript{24} and coworkers have also documented a few cases of non Hodgkin lymphoma in their studies.

No case of Hodgkin lymphoma or Kaposi sarcoma was identified in the present series. Similarly none of the studies conducted in India have documented nodal Kaposi sarcoma.

6. Conclusion

Lymph nodes biopsy is a valuable tool in the evaluation of HIV positive patients to identify the cause of lymphadenopathy. In addition to providing evidence of infectious disease like tuberculosis, it is of value even in those patients with reactive changes since the morphologic patterns encountered generally parallel their clinical course.

7. Source of Funding

None.

8. Conflict of Interest

None.
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