Etiological study of lymphadenopathy in HIV-infected patients in a tertiary care hospital

ABSTRACT

Introduction: Human immunodeficiency virus (HIV) infection has become a global pandemic. Persistent generalized lymphadenopathy (PGL) is a very common manifestation of HIV infection. Moreover, different opportunistic infections such as tuberculosis (TB) and malignancies may present with lymphadenopathy. Mycobacterium avium complex (MAC) infection is most common with a cluster of differentiation (CD)4+ count ≤ 50 cells/µL. Fine-needle aspiration cytology (FNAC) offers a simple and effective modality for obtaining a representative sample of the material from lymph nodes, permitting cytological evaluation and other investigations.

Aims and Objectives: The aim of this study is to find out the different etiologies of lymphadenopathy in HIV-infected patients and to establish a possible correlation with CD4+ count.

Materials and Methods: A total of 100 HIV-infected patients having significant (>1 cm) extranodal lymphadenopathy were studied in 1 year at the Department of Pathology by FNAC and the stains used were Leishman–Giemsa, Ziehl–Neelsen (ZN), Papanicolaou, and Gram stains. For tubercular culture, Löwenstein–Jensen (LJ) medium was used. CD4+ count was done by flow cytometer.

Result: The present study revealed four types of cytomorphological variants in lymphadenopathy cases by FNAC, which include: Reactive hyperplasia and caseation necrosis; caseation necrosis and ill-formed granuloma; well-formed granuloma without any necrosis; and non-Hodgkin lymphoma (NHL). The highest acid-fast bacilli (AFB) positivity was among the patients showing caseation necrosis. Tubercular culture in LJ media turned out as a more sensitive method for diagnosis than routine ZN staining. The 2 cases that showed well-formed epithelioid granuloma without any necrosis turned out to be histoplasmosis and cryptococcosis, respectively. In this study, we found 2 cases of NHL. The study also revealed that caseation necrosis and AFB positivity along with opportunistic infections increases with decreased CD4+ count.

Key words: Cluster of differentiation (CD)4+ count; human immunodeficiency virus (HIV); lymphadenopathy; tuberculosis (TB)

Introduction

Human immunodeficiency virus (HIV) infection and its resultant acquired immune deficiency syndrome (AIDS) have become a global pandemic.[1] HIV disease can be divided on the basis of immunodeficiency into an early stage [cluster of differentiation (CD)4 > 500/µL], an intermediate stage (CD4 = 200-500/µL), and an advanced stage (CD4 < 200/µL).[2,3] Most AIDS-defining opportunistic...
infections and malignancies occur in the advanced stage of the disease.[4,5]

Persistent generalized lymphadenopathy (PGL) is a very common manifestation of HIV infection. Moreover, different opportunistic infections such as tuberculosis (TB), toxoplasmosis, disseminated fungal infections, atypical mycobacterial infections, *Cytomegalovirus* infection, and malignancy such as non-Hodgkin lymphoma (NHL) may present with lymphadenopathy.[6,7] *Mycobacterium avium complex* (MAC) infection is most common with CD4+ count ≤ 50 cells/μL.[8]

Fine-needle aspiration cytology (FNAC) offers a simple and effective modality for obtaining a representative sample of the material from lymph nodes, permitting cytological evaluation and other investigations. Hence the aim of this study is to find out the different etiologies of lymphadenopathy in HIV-infected patients and to establish a possible correlation with CD4+ count.[9-12]

Materials and Methods

A total of 100 patients who have been diagnosed with HIV previously, having significant (>1 cm) extrainguinal lymphadenopathy (clinical or radiological) irrespective of their treatment status with antiretroviral therapy (ART) were included in study over a period of 1 year (January 2010 to January 2011). In all of these patients, after pretest counseling of the individual, blood samples were tested for anti-HIV antibody by enzyme-linked immunosorbent assay (ELISA) test at integrated counselling and testing centre (ICTC) according to National AIDS control organisation (NACO) protocol. Posttest counseling of the individuals was also done. CD4+ count was done by flow cytometry. FNAC from lymph nodes was done with disposable 10 mL syringe and 22 gauge needle. Image guidance was used where necessary. Leishman–Giemsa, Ziehl–Neelsen (ZN), Papanicolaou, and Gram staining was routinely used in all the cases. Positive cases were subjected to culture in Löwenstein–Jensen (LJ) medium for TB and in Sabouraud dextrose agar (SDA) with chloramphenicol for fungal culture.

Results

Most of the HIV-seropositive patients belonged to the age group of 25-45 years (78%) and out of them 76% were males, while 24% were females.

In this study the most common presentation was axillary lymphadenopathy (84%), 26% of them with isolated axillary node and others with various combinations. Sixty percent of patients had cervical lymphadenopathy. Among them, 12% had isolated cervical lymphadenopathy. Fifty-two percent of patients presented with generalized lymphadenopathy (involving >3 noncontiguous lymph node sites).

The highest number of patients (72%) were at World Health Organization (WHO) clinical stage 4, while 24% in stage 1, stage 2, and stage 3 both contained 4% each in our study.

Eighty percent of patients had extrapulmonary TB, among whom lymphadenopathy was the commonest (68%). Twenty percent of patients had isolated tubercular lymphadenopathy and 36% had associated pulmonary TB [Table 1].

Twenty-eight percent patients had very low CD4+ count (<50/μL). The highest number of patients (36%) presented with CD4+ count in the range of 50-200/μL, the next most frequently encountered group had >350/μL count (20%), followed by 200-350/μL count (16%).

On cytomorphological examination, an equal number of patients showed caseation necrosis (40%) and reactive hyperplasia (40%). Sixteen percent showed both caseation necrosis and ill-formed granuloma. Only 2 patients showed well-developed granuloma without any necrosis. Two patients showed NHL [Table 2].

| Cytological examination          | Number of patients | AFB +ve | Percentage (%) |
|----------------------------------|--------------------|--------|---------------|
| Reactive hyperplasia             | 40                 | 2      | 5             |
| Caseation necrosis               | 40                 | 32     | 80            |
| Caseation necrosis and ill-formed epithelioid cell granuloma | 16 | 10 | 62.5 |
| Epithelioid cell granuloma       | 2                  | 0      | 0             |
| NHL                              | 2                  | 0      | 0             |
| Total                            | 100                | 44     |               |

Table 1: Distribution of patients according to manifestation of tuberculosis

Table 2: Distribution of patients according to cytomorphological findings and ZN staining
granuloma (5 out of 5) showed the highest number of AFB-positive cases (80%), followed by caseation necrosis only (32 out of 40). In addition, 2 cases of reactive hyperplasia (out of 27) were AFB-positive [Table 2].

Out of 100 cases, only 76 cases were put to culture in LJ media. Out of 76 cases, 60 showed culture positivity. Among the reactive hyperplasia group (27 cases out of 40), 13 cases who did not show any AFB on ZN-stained smear showed culture positivity. All the cases of caseation necrosis (40 out of 40 were put to culture) and those with caseation-like necrosis and ill-formed epithelioid granuloma (5 out of 16 were put to culture) showed culture positivity. Two cases of epithelioid granuloma without necrosis showed growth on SDA medium [Table 3].

Out of the 60 cases that showed culture positivity, 56 showed growth after 8 weeks, while 4 of them showed growth within 3 weeks. We compared the cytological features, CD4+ count, morphology of the mycobacteria as well as the colony characteristics of the growth, and came to a provisional diagnosis of MAC. Confirmatory diagnosis can only be arrived at by molecular diagnostic techniques, but the features helpful in distinguishing MAC were: absence of granuloma in a necrotic background; small, deeply stained acid-fast coccobacilli in ZN stain; smooth, discrete, dull-white colonies; and absence of pigment production in LJ medium. The CD4 count was <50/µL in all these patients.

Reactive hyperplasia was highest (70%) among the patients having CD4+ count >350/µL. Reactive hyperplasia was significantly higher in number with the higher CD4+ count (P = 0.047). On the contrary, caseation necrosis was most commonly seen (71%) in patients having CD4+ count <50/µL.

Regarding AFB positivity, the maximum number (71.43%) AFB-positive cases were seen in a group of CD4+ count <50/µL. The lowest incidence (20%) was seen in CD4+ count >350/µL [Table 4].

There was significant association between AFB positivity and low CD4+ count (P = 0.017).

Discussion

HIV being a lymphotropic virus, lymphadenopathy is a very common manifestation of HIV infection.[11-13] Apart from other causes such as TB, opportunistic infections and NHL, HIV itself can cause reactive follicular hyperplasia, which manifests as PGL.[14-16] FNAC being a minimally invasive, cost-effective, and rapid procedure, it was used as a diagnostic procedure to determine the cause of lymphadenopathy in these patients.[11-13,17]

This study was done on 100 HIV-seropositive patients and most of them belonged to the age group of 25-45 years and the male-to-female ratio was 3:1, which was similar to observations by NACO.[18] The commonest site of lymph node involvement was the axilla, followed by cervical and generalized lymphadenopathy.[2,3,19] Eighty cases presented with extrapulmonary TB and most of them presented with peripheral lymphadenopathy. Thirty-six cases presented as pulmonary TB, which is similar to the observations by Mandal et al. and Rupali et al.[20,21]

The present study revealed four types of cytomorphological variants in lymphadenopathy cases by FNAC, which include reactive hyperplasia and caseation necrosis in an equal number of cases (40%), caseation necrosis and ill-formed

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**Table 3: Distribution of patients according to cytomorphological features and tubercular culture**

| Cytological examination | Number of patients put to culture (76) | AFB+VE in ZN-stained smears | TB culture in LJ-media +ve (60) | Fungal culture in SDA media +ve |
|-------------------------|----------------------------------------|-----------------------------|---------------------------------|--------------------------------|
| Reactive hyperplasia (40) | 27                                      | 2                           | 15                              | 0                              |
| Caseation necrosis (40)  | 40                                      | 32                          | 40                              | 0                              |
| Caseation necrosis and ill-formed epithelioid cell granuloma (16) | 5                                      | 5                           | 5                               | 0                              |
| Epithelioid cell granuloma | 2                                      | 0                           | 0                               | 2                              |
| NHL (2)                  | 0                                      | 0                           | 0                               | 0                              |

**Table 4: Distribution of the patients according to CD4+ count and FNAC findings**

| CD4+ count | No of patients | Reactive hyperplasia (%) | Caseation necrosis (%) | Caseation necrosis+ ill-formed granuloma (%) | Granuloma (%) | NHL | AFB +ve (%) |
|------------|----------------|--------------------------|------------------------|----------------------------------------------|---------------|-----|-------------|
| 0-50       | 28             | 4 (14.3)                 | 20 (71)                | 4 (14.3)                                     | 0             | 0   | 20 (71.43) |
| >50-200    | 36             | 14 (38.89)               | 12 (33.33) k3          | 10 (27.77)                                   | 0             | 0   | 16 (44.44) |
| >200-350   | 16             | 8 (50)                   | 4 (25)                 | 1 (6.25)                                     | 2 (12.5)      | 1   | 4 (25)     |
| >350       | 20             | 14 (70)                  | 4 (20)                 | 1 (5)                                        | 0             | 1   | 4 (20)     |
| Total      | 100            | 40                       | 40                     | 16                                           | 2             | 2   | 44          |
granuloma in 16% cases, well-formed granuloma without any necrosis in 2% cases, and NHL in 2% cases.

In this study, the highest AFB positivity was among the patients showing caseation necrosis plus ill-formed granuloma (100%), followed by caseation necrosis only (80%). Two cases of reactive hyperplasia showed AFB positivity. The yield of AFB was highest in necrotic lesions. Bezabih et al., Handa et al., and Gupta et al. have reported ZN positivity of 37.4-59.4% in aspirates from tubercular lymphadenitis.[22,23]

Tubercular culture in LJ media is taken as the gold standard for diagnosis. Out of 100 patients, only 76 cases were put to culture. For other cases, FNAC material was not sufficient for inoculation and repeat aspirations could not be performed due to patient denial. Sixty cases showed culture positivity. All the cases of caseation necrosis showed culture positivity. All the 5 cases of caseation necrosis plus ill-formed granuloma that were put to culture showed culture positivity. Thirteen patients having reactive hyperplasia and 8 patients having caseation necrosis, who did not show any AFB in ZN-stained smears, were positive for culture, thus showing culture as a more sensitive method for diagnosis.[8,9,22,23]

Among the culture-positive cases, 4 cases showed growth within 3 weeks of inoculation. On further comparison of their FNAC smears, morphology in ZN-stained smears, colony characters in LJ media and CD4+ count, a provisional diagnosis of MAC has been made for those 4 cases, though definitive diagnosis requires molecular diagnostic techniques. Nightingale et al. and Johnson et al. also found the MAC as one of the most common pathogens causing bacteremia in HIV-positive patients.[7,8]

Two cases that showed well-formed epithelioid granuloma without any necrosis turned out to be histoplasmosis and cryptococcosis, respectively. The former was confirmed after doing a periodic acid schiff (PAS) staining of the FNAC smear from its distinctive morphology. The other case when subjected to negative staining in India ink preparations showed halos around yeast cells, which lead us to a diagnosis of Cryptococcus species in this case, which is very common in immunocompromised individuals as was proposed by the study of Fernendez et al.[19]

In this study we found 2 cases of NHL. NHL of B-cell lineage is most common lymphoma in HIV-infected patients.[16] The most common one is diffuse large B-cell lymphoma followed by Burkitts lymphoma. In our present study, we found 2 cases of NHL, but confirmation by histopathology and immunohistochemistry could not be done in these cases.

Kumarguru et al. showed 46.32% cases of reactive hyperplasia and 41.55% cases of tubercular lymphadenopathy.[9] Studies conducted by Shenoy et al. in Mangalore (50%), Saikia et al. in Chandigarh (32%), and Jayaram et al. in Malaysia (53.84%) also observed tuberculous lymphadenitis as a common lymph node lesion.[11,12]

Out of 100 patients, 28 had very low CD4+ count (<50/μL). Highest number of patients presented with CD4+ count in the range of 50-200 cells/μL. Reactive hyperplasia was significantly higher in number, with higher CD4+ counts (P = 0.047). On the contrary, caseation necrosis was higher in number, with lower CD4+ counts. There was significant association between AFB positivity and low CD4+ count (P = 0.017), which was in accordance with the study of Gautam et al. and Jones et al.[2,3]

Conclusion

HIV-infected patients can present with a variety of causes for lymphadenopathy, extrapulmonary TB being the most common among them. Every HIV-infected patient with significant lymphadenopathy should undergo FNAC study as it can help in etiological diagnosis of the lesion. Caseation necrosis is highly diagnostic of TB. The sensitivity of the test is increased when both ZN staining and culture in LJ media are used for diagnosis. Caseation necrosis and AFB positivity increases with decreased CD4+ count.

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Conflicts of interest
There are no conflicts of interest.

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Announcement of New Award Instituted by Indian Academy of Cytologists in 2016 for Best Published Article in Journal of Cytology

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   (a) Publication should be in Journal of Cytology.
   (b) Publication should be during the past one year and shall comprise of papers from Issues 3 and 4 of previous year and Issues 1 and 2 of current year.
   (c) Ahead of print articles shall not be considered for the award.
   (d) Publication should be an original article.
   (e) The awardee shall be the first author of the published paper.