CLINICO-PATHOLOGICAL CORRELATION OF LYMPHADENOPATHY IN HIV POSITIVE PATIENTS
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ABSTRACT: BACKGROUND: Lymphadenopathy is common in HIV infected individuals as lymphoid tissue is a major target and reservoir of the Human immunodeficiency virus (HIV) as well for opportunistic infections. AIM: Clinical assessment of HIV positive patients with lymphadenopathy and its correlation with lymph node fine needle aspiration cytology (FNAC) and CD4 count. STUDY DESIGN: Cross sectional study of 100 HIV positive patients. MATERIALS AND METHODS: Clinical profile of 100 HIV positive patients with lymphadenopathy was taken. FNAC of the largest lymph node was done. Smears collected were stained by different staining techniques like papanicolou staining, H& E staining, Giemsa staining and AFB as well as PAS staining. FNAC finding was compared with the CD4 count. RESULTS: Of the 100 patients studied, maximum number of cases was reported in the age group of 31 to 40 years. Majority of the patients were males (81%). Most common presentations were fever in 71%, weakness in 71%, weight loss in 53% and lymph node swelling in 23% of patients. Most common site of lymph node involvement was cervical. FNAC of lymph nodes revealed that maximum number of cases had tuberculosis lymphadenitis (59%), followed by reactive hyperplasia (37%), non-Hodgkin's lymphoma (2%), cryptococcosis (1%) and metastasis (1%). The mean value of CD4 count was 461 for reactive hyperplasia, 294 for TB lymphadenitis, and 318 for others. Multiple comparisons of FNAC with CD4 count, revealed significant p-value between Tubercular lymphadenitis and Reactive hyperplasia. CONCLUSION: Low CD4 count is associated with Tubercular lymphadenitis and higher CD4 count with Reactive hyperplasia.

KEYWORDS: HIV, lymphadenopathy, FNAC, CD4 count.

INTRODUCTION: India has a population of one billion, around half of whom are adults in the sexually active age group. The first case of acquired immune deficiency syndrome (AIDS) case in India was detected in 1986 and since then HIV infection has been reported in all states and union territories.

The total number of people living with HIV/AIDS in India is estimated at 23.9 lakh in 2009. Children less than 15 years account for 3.5 per cent of all infections, while 83 per cent are in the age group 15-49 years.[1]

The spread of HIV in India has been uneven. Although much of India has a low rate of infection, certain places have been more affected than others. HIV epidemics are more severe in the southern half of the country and the far north-east. The highest estimated adult HIV prevalence is found in Manipur (1.40%), followed by Andhra Pradesh (0.90%), Mizoram (0.81%), Nagaland (0.78%), Karnataka (0.63%) and Maharashtra (0.55%).[1]
The virus of HIV infects lymphocytes and during the progression of this infection it has a devastating effect on the immune system of the individual. The chronic progression of HIV infection results in the acquired immunodeficiency syndrome (AIDS). Due to the fact that lymphocytes are the target cells for the infection, the symptom of enlarged lymph nodes (LN). Lymphadenopathy (LA) is the earliest and one of the most common and consistent symptoms during the HIV infection. This may be due to the presence and effects of HIV. Lymphadenopathy may also be a manifestation of opportunistic infections or lymphoid malignancy developing in an immune deficient individual.

The emphasis of our study was to identify and describe these particular, distinctive infections that usually accompany HIV. The clinical aspects of LA in the different associated infections were one of the major goals of this study. The possibility of the detection of hidden HIV associated infections by means of FNAC as diagnostic method and comparing with CD4 count is another focus of our study.

MATERIALS AND METHODS: In the present study, 100 cases who tested HIV positive according to NACO guidelines [1] and had enlargement of non-inguinal lymph nodes, size ≥1cm were included. All the patients underwent clinical evaluation FNAC was done from the largest non-inguinal lymph node with informed consent. A total of 100 lymph node aspirations were performed and aspirates were deposited on to the slides. Subsequently air dried, alcohol fixed and heat fixed smears were made. For each case haematoxylin and eosin, Wrights and Ziehl-Neelson staining were routinely done. However Grocott-Gomori Methenamine silver, periodic acid Schiff, mucicarmine, Auramine and Rhodamine and grams staining were done in selected cases. CD4 count of these patients was done by flow cytometry and correlated with the FNAC finding.

RESULTS: A total of 100 HIV positive patients with lymphadenopathy were taken into consideration.

AGE AND SEX DISTRIBUTION: Out of 100 cases studied, 81 patients were male while 19 patients were female, male to female ratio being 4.26:1. Most of the cases were found in 31-40 years of age group (41%), 34% were in the age group 21-30 years and 19% in age group of 41-50 years.

SYMPTOMATOLOGY: Most common presentations were fever in 71%, weakness in 71%, weight loss in 53% and LN swelling in 23% of patients. (FIGURE No. 1)

DURATION OF DISEASE: Most of the cases were newly diagnosed (59%), 9% of patient had the disease for ≤1yr, 21% of patient had the disease for 2-4yrs and 11% of patient for 5-10yrs.

LYMPHADENOPATHY: SITE OF LYMPHNODE INVOLVEMENT: Most common site of lymphnodes involved in our study was cervical (70%), the other sites of involvement were axillary (38%), supraclavicular (34%), inguinal (28%), submandibular (8%) and submental (2%). Generalized lymphadenopathy was seen in 40% of the study population.
SIZE OF LYMPHNODE: In our study 72% of patients had Lymphnode size ≤2cm and 28% of patients had lymph node size of >2cm.

LYMPHNODE MATTING: In our study lymphnode matting was seen in 20% of patients.

FNAC FINDING: Of the 100 patients studied, FNAC results of 59 patients (59%) showed TB lymphadenitis and 37 patients (37%) showed reactive lymphadenitis. Other causes of lymphadenopathy amounted to 4% (Non-Hodgkin’s lymphoma-2, Cryptococcal lymphadenitis-1 and metastasis-1).

CORRELATION OF FNAC WITH CD4 COUNT: The absolute CD4 count correlated well with the FNAC findings. As evidenced by highly significant p-value (<0.0001).

The mean value of CD4 count was 461 for reactive hyperplasia, 294 for TB lymphadenitis, and 318 for others (337 for NHL and 448 for cryptococcal lymphadenitis). (TABLE No. 1)

MULTIPLE COMPARISONS OF FNAC WITH CD4 COUNT: Multiple comparisons of FNAC with CD4 count, revealed significant p-value (<0.0001) between Tubercular lymphadenitis and Reactive hyperplasia (Post Hoc analysis done with Bonferroni correction). It infers that low CD4 count is associated with Tubercular lymphadenitis and higher CD4 count with Reactive hyperplasia. (TABLE No. 2)

DISCUSSION: According to Medical Microbiology,[2] HIV is a member of the genus Lentivirus belonging to Retroviridae family. Lentivirus means ‘slow’ virus and this name is given to the group due to the long interval between the initial infection and the onset of severe symptoms.[3]

Two genetically distinct viral types have been identified. HIV-1 which is present in Europe, Asia, America and Central Africa and HIV-2 which is only situated in the Western Africa.[4]

The process of the selection of target cells that are infected by HIV is based on the recognition of CD4 receptors on the host cells surface by the virus. The main function of the CD4 ‘T-helper’ cells is activation of other cells of the immune system such as B-lymphocytes and cytotoxic T cells.[5]

Lymphadenopathy is one of the profound and persistent signs during the progression of the HIV infection. The HIV virus infects primarily the CD4 lymphocytes therefore the lymph nodes are commonly involved during all the stages of the infection.[6]

The syndrome of ‘persistent generalized lymphadenopathy’ was described as one of the first symptoms of HIV infections. This type of LA is present throughout the life of the patient. The lymphnode swelling in atypical areas is regularly present such as supracondylar and submandibular regions.[6]

Enlargement of the lymph nodes that persists for at least three months in at least two extrathoracic sites is defined as persistent generalized lymphadenopathy and is common in patients in the early stages of HIV infection. Other causes of generalized lymphadenopathy in HIV infected patients include Kaposi’s sarcoma, CMV, toxoplasmosis, tuberculosis, cryptococcosis, syphilis and lymphoma.[7]
In this study the relation between HIV and the following infections are described: Tuberculosis (TB), Non tuberculous mycobacteriosis, Cytomegalovirus, Pneumocystis jirovecii, Fungi, Toxoplasmosis, Mycoplasmosis, Cryptococcosis and Clostridiosis.

Pulmonary, extra pulmonary tuberculosis and mycobacteremia becomes more common when the level of the immunosuppression increases and HIV-infection progresses.\[8\]

Non-tuberculous mycobacteria have been reported to cause localized or disseminated disease depending on local predisposition and/or degree of immune deficit. In AIDS patients the manifestations may range from localized to disseminated disease.\[9\]

Mycobacterium avium complex (MAC) isolates have been reported to be the cause of lymphadenitis. M. scrofulaceum, whose distribution in nature closely resembles that of M.avium, has also been found to be a common cause of cervical lymphadenitis in western countries\[9\] and has been reported from India as well.\[10\] M.bohemicum, M.szulgai and M. interjectum, a new species resembling M.scrofulaceum have been isolated from cases of lymphadenitis.\[11\]

The main symptoms of CMV are cough, fever and dyspnoea and are present for 2-4 weeks. LA may be present but is not a common symptom. A study made by the group of Salomon et al. reports hilar and mediastinal lymphadenopathy in 2 cases from 18 patients with HIV associated CMV pneumonia.\[12\]

A number of others opportunistic infections have an increased incidence in immunosuppressed HIV positive individuals. As a rule these infections flourish when the counts of CD4 T-lymphocytes are below 200 cells per μl in the AIDS phase. They can also be the reason to the persisting LA that is specific to this terminal stage of the disease progression. When the immune system begins to show a lack of its own function normal commensal microorganisms begin to invade the body. Some species of fungi, Candida and Aspergillus and protozoal parasite like toxoplasma and cryptococcosis are examples.

There is an association between a group of tumours and HIV-infection and are therefore linked to the level of immunosuppression. The most common AIDS associated cancers are Kaposi sarcoma and non-Hodgkin lymphoma (NHL), their rates are increased after even a modest immune suppression. Hodgkin lymphoma (HL) is also associated with HIV but it is more frequently seen when the immune deficiency is more profound. There are also several unusual lymphoproliferative entities that have an increased incidence in HIV positive cases like primary effusion lymphoma, Castleman’s disease, EBV associated lymphoproliferative disorder, T-cell lymphomas and primary central nerve system lymphoma.\[13\]

We studied 100 HIV positive patients in the age group 15 years to 65 years. The commonest age group to be affected was 31-40 yrs (41%) followed by 21-30 yrs (34%). We found that males were more commonly affected by HIV infection than females with the male: female ratio of 4.26:1. Further, cervical lymph nodes were the most common affected site. Shenoy et al.\[14\] in their study noticed that male: female ratio was 5:1 and the maximum numbers of the patients were present in 25-30 years of age group with cervical group of lymph nodes being the most commonly affected site. Vanisri et al.\[15\] also noted cervical node group as the most common site involved. However, Satyanarayana et al.\[16\] reported axillary node involvement being more common in their study.
Reactive lymphadenitis and Mycobacteria infection are the 2 most common findings in the almost all studies. Shenoy et al. and Vanisri et al. found the most common finding to be tuberculosis as in our study. Whereas Bates et al. Reid et al. Guru et al. and Satyanarayana et al. noticed that reactive lymphadenitis was the most common cytological finding.

We found lymphoma to be the aetiology of lymphadenopathy in 2% of cases (2 cases of NHL) which was comparable to the study done by Satyanarayana et al. (2.6%) and Vanisri et al. (2.7%). Whereas few other studies noted a higher number of cases of lymphoma than in the present study (Bates et al. noted 4%, Reid et al. noted 9% and Shenoy et al. noted 8.9%).

Kaposi sarcoma was not found in any case in the present study as well as in other studies conducted by Indian authors (Satyanarayana et al. and Vanisri et al.) but studies in western countries (Bates et al. and Reid et al.) noted few number of cases of Kaposi sarcoma.

Other findings noted in the present study were, one case of cryptococcal lymphadenitis and one case of metastatic lymphadenitis (PDC). This matched with the study conducted by Bates et al. and satyanarayana et al. (one case of Cryptococcal lymphadenitis each in their study).

In the present study, median CD4 count in Reactive lymphadenitis was the highest 426 among all the other lesions, followed by TB lymphadenitis 286. Study conducted by shobhana et al. reveals median value of CD4 count to be 672 and 212 cells/µL in case of reactive hyperplasia and TB lymphadenitis respectively.

It is generally considered that lymphoma is associated with low CD4 count, where the CD4 count may fall below 200cells/µL, but our observation in the present study did not corroborate well with the description (In the present study, CD4 count in 2 cases of NHL was 400 and 267 cells/µL).

**CONCLUSION:** FNAC is one of the safe, sensitive as well as specific diagnostic test for investigation of the cause of lymphadenopathy in HIV patients. Procedure is rapid and easily performed it obviates the need of excision, guides subsequent therapy or observation and provides definite guidelines for management.

Correlation of lesions with CD4+ T lymphocyte counts provides information about immune status and stage of the disease.

Low CD 4 count is associated with Tubercular lymphadenitis and higher CD 4 count with Reactive hyperplasia.

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| FNAC       | No. of patients | CD 4 COUNT | ANOVA F | P Value |
|------------|-----------------|------------|---------|---------|
|            | Min  | Max  | Mean   | Std deviation | Median |         |        |
| TB LYMP    | 59   | 36   | 579    | 294.88     | 128.281 | 286.00  | 17.085 | <0.0001 |
| REAC HYP   | 37   | 202  | 808    | 461.89     | 150.233 | 426.00  |         |         |
| OTHER      | 4    | 158  | 448    | 318.25     | 131.434 | 333.50  |         |         |
| TOTAL      | 100  | 36   | 808    | 357.61     | 157.619 | 367.50  |         |         |

**TABLE No. 1: Correlation of FNAC with CD4 count**

| (I)FNAC | (J)FNAC | Mean Difference (I-J) | Std. Error | P value |
|---------|---------|-----------------------|------------|---------|
| TB LYMP | REAC HYP| -167.011*             | 28.715     | <0.0001 |
|         | OTHER   | -23.369               | 70.749     | 1.000   |
| REAC HYP| TB LYMP | 167.011*              | 28.715     | <0.0001 |
|         | OTHER   | 143.642               | 72.072     | 0.147   |
| OTHER   | TB LYMP | 23.369                | 70.749     | 1.000   |
|         | REAC HYP| -143.642              | 72.072     | 0.147   |

**TABLE No. 2: Multiple comparisons of FNAC with CD4 count**
Dependent variable: CD4 count Bonferroni

*The mean difference is significant at the 0.05 level.

| Diagnosis      | Bates et al.[17] n=27 | Reid et al.[18] n=65 | Sathyanarayana et al.[16] n=196 | Vanisri a et al.[15] n=36 | Guru et al.[19] n=231 | Present study n=100 |
|----------------|-----------------------|----------------------|---------------------------------|--------------------------|-----------------------|---------------------|
| Mycobacteria   | 22%                   | 15%                  | 34.2%                           | 58.3%                    | 41.55%                | 59%                 |
| Reactive       | 41%                   | 51%                  | 42.3%                           | 36.1%                    | 46.32%                | 37%                 |
| Lymphoma       | 4%                    | 9%                   | 2.6%                            | 2.7%                     | 1.73%                 | 2%                  |
| Kaposi’s sarcoma| 15%                   | 2%                   | -                               | -                        | 1.29%                 | -                   |
| other          | 18%                   | 23%                  | 23.5%                           | 2.7%                     | 1.72%                 | 2%                  |

**TABLE No. 3: Comparison of FNAC finding with other studies**
Figure No. 1: Symptomatology

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