STUDY OF PREVALENCE OF GRANULOMATOUS LYMPHADENITIS IN INDIVIDUALS AFFECTED WITH HIV INFECTION

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ABSTRACT

BACKGROUND

Prevalence of Granulomatous lymphadenitis in human immune deficiency virus infection is increasing drastically in developing countries, particularly in India. In the present study, an attempt has been made to find an association of Granulomatous lymphadenitis in human immune deficiency virus infection in humans.

The aim of this study is to correlate the FNAC finding with clinical, radiological and morphological patterns like correlation with findings and the CD4 count, relation of Mantoux to lymph nodes and relation with x-ray findings.

MATERIALS AND METHODS

A descriptive study done in 100 adult patients, aimed to study the prevalence of tuberculous lymphadenitis by cytological patterns in lymph nodes of HIV sero-positive patients by collecting and studying material through FNAC and clinical importance by Mantoux and x-ray findings. Patients attending Government General Hospital, ACSR GMC, Nellore who was not on ATT or ART were chosen for the study.

RESULTS

Various lesions were observed in this study. Granulomatous lymphadenitis was observed in 74 cases, reactive lymphadenitis was seen in 22 cases and acute suppurative lymphadenitis was seen in 3 cases. One case has shown secondary deposits of squamous cell carcinoma.

CONCLUSION

FNAC is a relatively inexpensive initial investigative technique important for assessing the cause of lymphadenopathy in HIV patients; allows a rapid diagnosis obviating the need for surgery and enabling swift treatment to be undertaken where necessary. Universal safety precautions must be strictly followed. The method is accurate, cost effective and acceptable to patients. TB is the major opportunistic infection, which could be detected in both symptomatic and asymptomatic individuals and even in tiny nodes. There was strong association with Mantoux and x-ray for diagnosing Granulomatous lesions.

KEYWORDS

Granulomatous Lymphadenitis, HIV.

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BACKGROUND

AIDS is a disease caused by the retrovirus human immune deficiency virus (HIV) and characterised by profound immune suppression that leads to opportunistic infections, secondary neoplasms and neurologic manifestations.¹ The incidence of human immunodeficiency virus (HIV) infection is increasing in Asia, particularly in Indian subcontinent.² Acquired immunodeficiency syndrome (AIDS) is known to be caused by lymphotropic retrovirus first described by French investigators in United States. AIDS was recognised in 1981. It has become clear that this syndrome represents the most

severe form of a broad-spectrum disease.³ AIDS is a fatal illness that breaks down the body's immunity and leaves the victim vulnerable to life-threatening malignancies.⁴ In India, the human immunodeficiency virus (HIV) epidemic is now a decade old and within this short period, it has emerged as one of the most serious public health problems in our country.⁵

Great advances have been made in the treatment of patients with HIV infection.⁶ These advances are of critical value in providing best opportunity for patients to live long or healthy life despite the presence of HIV infection. Lymphadenopathy is one of the earliest manifestations of HIV infection.⁷ In developing countries like India with a huge population and socioeconomic constraints, there is a great need for a simple investigative technique for HIV infected lymphadenopathy cases.⁶ The role of fine needle aspiration cytology in the evaluation of lymphadenopathies is well known.⁷

FNAC has become the primary investigative procedure for mass lesions on HIV-positive patients, particularly in the assessment of lymphadenopathy.⁸ It is simple, cost effective, low risk procedure. The procedure is rapid, easily performed

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and in many cases, obviates excision while guiding subsequent therapy or observation.

MATERIALS AND METHODS

Study Design

It is a Descriptive study.

Study Setting

Study was conducted at general medicine outpatient department, pulmonology outpatient department, Govt. General Hospital, Nellore.

Sample Size

It was calculated by using the following formula.

$$[n = 4pq / d^2] \quad p = 50, q = 50, d = 10$$

Where n= sample size, p= prevalence in %, q= 100-p, d= precision.

Aspirations were performed either in outpatient clinic or in inpatient wards. The patients were informed about the procedure and consent was taken. A 23-gauge needle and 10 mL disposable syringe was used on largest lymph node with several passes with standard precautions. FNAC cytology material was spread directly on to glass slides and fixed in isopropyl alcohol and smears were stained with haematoxylin and eosin.

Ziehl-Neelsen stain was done in clinically suspected tuberculosis cases. CD4 count was done by flow cytometry. Examination of sputum for AFB, x-ray chest and Mantoux were done when needed.

Lymph Node Sites/ Regions Selected for FNAC

Neck nodes- Anterior cervical, posterior cervical, submandibular axilla- lateral, anterior, posterior, central and apical inguinal.

Technique of Fine Needle Aspiration Cytology

FNAC technique employed in this project was as per the guidelines described in the text book- "Fine Needle Aspiration Cytology" 4th Edition by Svante R Orell, et al.

Principle

Aspiration of cellular material from target lymph nodal masses applying negative pressure by a syringe.

Procedure

Patient was placed in a comfortable lying or sitting position and the swelling was palpated to identify the size, consistency, depth and its relation to surrounding structures. The skin overlying the swelling was cleaned with spirit swab. No local anaesthesia was used.

A 10-mL disposable syringe was attached to a 23-gauge disposable sterile needle and inserted into the mass after the swelling was fixed with the index and thumb of left hand. The plunger of the syringe was retreated once the needle was in the swelling to create a negative pressure in the syringe and the needle lumen without using syringe holder.

The needle was moved back and forth several times within the target tissue and in various directions to get the greatest possible cellular yield. Throughout the procedure, negative pressure was maintained in the syringe. Admixture with the blood tends to be less if the needle is moved along

the same track rather than in various directions. The hub of the needle should be observed for any material or blood. It is not necessary to see the material in the hub of the needle, but once the material is observed the aspiration was stopped.

The needle pressure in the syringe must be released before the needle is withdrawn from the target tissue by gently releasing the plunger to avoid contamination by material aspirated during withdrawal. The needle was withdrawn from the lesion and pressure was applied at the puncture site with sterile gauze pad for at least 3 minutes in order to prevent haematoma formation. The ideal aspirate was a creamy consistency due to high cellular yield in a small amount of fluid.

If the target tissue is cystic, entire fluid is aspirated. The aspirated fluid centrifuged for 5 minutes at 1500 rpm within 2 hours after removal. Smears are made from the sediment and fixed in 95% isopropyl alcohol. Those slides were never allowed to become dry. Smears are to be stained by haematoxylin and eosin. The target lesion is palpated again to observe if residual mass is present and FNAC is done once again.

Smear Preparation

If the material was solid or semi-solid in nature, optimal, uniform in the spread smears were obtained by superimposing two glass slides with expressed cellular materials sandwiched between them, exerting gentle pressure for a few seconds and then rapidly pulling the slides apart horizontally resulting into two separate spread smears. If the aspirated material is fluid or diluted with blood, smears were made as that of peripheral smears. Prepared smears were fixed in 95% isopropyl alcohol for 30 minutes and stained with haematoxylin and eosin.

Staining Technique

Haematoxylin and eosin stain was used. Nuclear details like nucleoli, nuclear chromatin and nuclear membrane were made out clearly with H and E technique.

Tuberculin Skin Test/ Mantoux Test

In this test 0.1 mL of PPD containing 5TU is injected intradermally on the flexor aspect of the forearm with a tuberculin syringe raising a wheal. Injection is given between the layers of skin and not subcutaneously. The site is examined after 48 - 72 hrs. and induration measured at its widest point transversely to the long axis of the forearm. Induration of diameter 10 mm or more is considered positive, 5 mm or less is negative and 6 - 9 mm is equivocal.

Ziehl-Neelsen Staining Procedure

The smear is covered with strong carbol fuchsin and gently heated to steaming for 5 - 7 min without letting the stain boil and become dry. Heat should be applied intermittently and slide is then allowed to cool.

The slide is then washed off with water and decolourised with 20% H₂SO₄ till no more stain comes off. Counter stain with Loeffler's methylene blue or malachite green for 2 mins. the stain is then washed off with water and allowed to dry in air. The smear is examined under oil immersion objective lens. Acid fast bacilli are seen as bright red slender rods against blue background.

Statistical Analysis of Data

It was only a descriptive study; the aim was restricted to calculation of proportions. No other statistical tests were performed.

RESULTS

A total of 100 adult cases of HIV sero-positive patients, who were not subjected to ATT or ART therapy were included in the present study. Fine needle aspiration of lymph node (s) was done and the smears were stained with routine cytological stains and special stains wherever needed. A cytomorphological study was done in each case.

Majority of lymph nodes chosen were from cervical area, the other sites being axillary and inguinal.

Various lesions were observed in this study. Granulomatous lymphadenitis was observed in 74 cases reactive lymphadenitis was seen in 22 cases, acute suppurative lymphadenitis was seen in 3 cases. One case has shown secondary deposits of squamous cell carcinoma.

Count of Report	Mantoux		Grand Total
	Negative	Positive	
Granulomatous Lymphadenitis	14	60	74
Reactive	22	0	22
Suppurative Lymphadenitis	3	0	3
Secondary Deposits	1	0	1
Total	40	60	100

Table 1. Table showing Results of Mantoux Test

Of the total 74 Granulomatous lymphadenitis patients, 60 cases show Mantoux test positive.

CD4 Count	Granulo matous	Reactive	Suppura tive	Secon dary	Total
>200	61	16	3	1	81
<200	13	6	0	0	19
Total	74	22	3	1	100

Table 2. Table showing CD4 Count in Relation to Lesions

Out of the total 100 cases, in 81 cases the CD4 counts were more than 200 and in 19 cases the CD4 counts were less than 200.

Count of Report	X-Ray		Grand Total
	Normal	Positive	
Granulomatous	41	33	74
Reactive	22	0	22
Suppurative Lymphadenitis	3	0	3
Secondary Deposits	1	0	1
Total	66	34	100

Table 3. Table showing X-Ray Findings Lesion Wise

Out of 74 Granulomatous lymphadenitis, 33 cases have positive x-ray findings.

DISCUSSION

Lymphadenopathy is one of the most common conditions encountered in clinical practice and its exact diagnosis poses problem to the clinicians as well as to the pathologists. Present study was carried out in the Department of Pulmonology, General Medicine and ART centre with collaboration of Department of Pathology, ACSR Govt. Medical College, Nellore, on HIV seropositive patients who are not on ATT/ART.

The present study of FNAC of lymph nodes of different sites with different lesions is found to be quite reliable. The percentage correlation of each type of lesion was calculated and it has been compared with the work of others, which have been mentioned in the following paragraphs.

Granulomatous Lesions

In the present study, out of 100 adult cases, 74 cases were due to granulomatous lesions. The diagnosis is based on presence of epithelioid cells which are plump, spindle-shaped cell with fusiform vesicular nucleus which appears as "Foot Print" appearance of Langerhan's giant cells are also encountered in cytology. Caseous necrosis with epithelioid granulomas was observed in 47 cases and granulomas without caseation was observed in 27 cases.

Only those cases in whom Ziehl-Neelsen stain positive were diagnosed as tuberculous granulomatous lesions (60 cases) and rest as chronic nonspecific granulomatous lesions (14 cases).

Tuberculous lymphadenitis was the most common lesion constituting 60 cases. Studies conducted by Guru et al⁹ (41.55%) in Belgaum, Shenoy et al¹⁰ (50%) in Mangalore, Saikia et al² (32%) in Chandigarh and Jayram et al⁷ (53.84%) in Malaysia also observed Tuberculous lymphadenitis as a common lesion.

However, Western studies conducted in California by Bottles et al¹¹ (17%) in Europe by Reid et al¹² (15%) and Martin-Bates et al¹³ (22%) demonstrated lower number of cases in comparison with the present study.

Reactive Lymphadenopathy

In the present study, out of 100 adult cases, 22% cases are presented with reactive lymphadenitis. Smears showed polymorphous cell population with mature lymphocytes and transformed lymphocytes (follicular center cells, monocytoid cells, neutrophils and tingible-body macrophages).

The reactive cytomorphological changes are sometimes seen in early cases of TB and Hodgkin's.

In the study conducted by Bottles et al¹¹ on patients with HIV and lymphadenopathy 50% of aspirates showed reactive hyperplasia.

Bates et al¹³ found reactive changes in 41% of HIV aspirates. Satyanarayana et al¹⁴ reported a reactive cytomorphological pattern in 16.4% of their cases of tuberculosis, which is lower than our study.

Acute Suppurative Lymphadenitis

In present study, 3% cases of HIV are presented with acute suppurative lymphadenitis. The aspirate was purulent. Smears show necrotic background with sheets of neutrophils and lymphocytes. ZN stain was negative in these 3% cases.

In the study conducted by Grossl et al¹⁵ mean CD4 counts observed in malignant lymphomas was 105/mm. In present study as we excluded patients on ATT/ART, the least CD4 count was 110/mm. This may be correlated.

In the present study, squamous cell carcinoma (metastatic) was identified in one case of HIV. Cytologically, smears show polyhedral pleomorphic cells with hyperchromatic nuclei arranged in sheets in the background of lymphocyte.

The study by Saikia et al² one case of squamous cell Ca (Metastatic) was diagnosed in HIV cases.

Mantoux Test

In present study, out of 74 granulomatous lymphadenitis 60 cases were positive for Mantoux and 14 cases were negative. In Vanisri et al¹⁶ out of 21 cases 10 cases are Mantoux positive.

CD4 Count in Relation to Lesions

In present study, CD4+ counts were more than 200 in 81 cases. Out of 81 cases 61 were granulomatous, 16 cases of reactive lymphadenitis, 3 cases were acute suppurative lymphadenitis and 1 case of secondary deposit metastases.

In remaining 19 cases CD4 counts were less than 200, out of which 13 cases were granulomatous inflammation, 6 were reactive lymphadenitis.

In the study conducted by Grossl et al¹⁷ CD4 counts mean in Acute suppurative inflammation, Granulomatous inflammation, Reactive lymph nodes, malignant lymphomas and suspicious for Kaposi sarcoma were 330, 145, 237, 105, 77/mm respectively.

If the lymph node is adequately sampled, one aspiration will yield enough material for diagnosis. In our study, all the samples are adequate.

Relation with X-ray Findings

Out of 74 Granulomatous lymphadenitis, 33 cases have positive X-ray findings.

CONCLUSION

- FNAC is a relatively inexpensive initial investigative technique important for assessing the cause of lymphadenopathy in HIV patients.
- Allows a rapid diagnosis obviating the need for surgery and enabling swift treatment to be undertaken where necessary.
- Universal safety precautions must be strictly followed.
- The method is accurate, cost effective and acceptable to patients.
- TB is the major opportunistic infection, which could be detected in both symptomatic and asymptomatic individuals and even in tiny nodes.
- AFB and other specific pathogens should be looked for even the cytological picture is scanty cellularity.

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