FINE NEEDLE ASPIRATION CYTOLOGY OF LYMPH NODES IN HIV INFECTED PATIENTS

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ABSTRACT

Lymphadenopathy is the commonest presentation in HIV positive individuals. Fine needle aspiration cytology of 196 HIV positive patients was studied during six monthly review. 75% patients in this study who were asymptomatic were detected to have lymphadenopathy during the surveillance. 82% had lymph nodes smaller than 1cm size. Lymphadenopathy at more than one site was observed in 46.8% cases. Commonest opportunistic infection noticed was tuberculosis (TB) in 34.2%. Cyto-morphologically reactive pattern with Acid fast bacilli (AFB) positivity was observed in 16.4% of TB cases. In 2.9% cases AFB were detected even in the tissue fluid. Negative images of AFB were observed in the macrophages in 3 cases. TB was detected with equal frequency in both asymptomatic and symptomatic groups. Axillary nodes pose problem due to deeper location. False positives were a case of dermatopathic lymphadenopathy and a case of Kimura's disease. False negatives include two cases of TB lymphadenitis. Pathogens should be looked for irrespective of cyto-morphology. Biopsy should be done to confirm cases of lymphomas. Fine needle aspiration cytology should be included in the protocol of six monthly review of HIV infected cases.

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KEY WORDS :AIDS; FNAC; HIV; Lymph node; Tuberculosis.

Introduction

Lymphoid tissue is a favourite target organ for the initial viral infection, subsequent opportunistic infections and Human Immunodeficiency Virus (HIV) associated neoplasms [1]. Fine needle aspiration cytology (FNAC) has a well established role in the diagnosis of lymphoid lesions. Acquired Immuno Deficiency Syndrome (AIDS) related lymphadenopathy has definite patterns like florid reactive hyperplasia, folliculolysis, explosive follicular hyperplasia, advanced lymphocytic depletion with or without abnormal regressively transformed germinal centers and vascular transformation. Though, FNAC can not clearly demarcate all these lesions, it has definite identifiable reactive patterns described and is useful in detecting specific infective aetiologies. Fine needle aspiration definitely involves lesser risk to the performer than the open biopsies. A retrospective study done by Burton F et al had indicated that open biopsy is not indicated in the HIV or AIDS patients especially with non tender or non-enlarging nodes, though it was the method of choice for diagnosing HIV infection before serological testing was freely available [2]. The literature available is mostly of the western world [1,3]. The presentation, clinical progress of the disease and the nature of opportunistic

infections in HIV positive and AIDS patients differ considerably in Indian setting. This study was undertaken with an aim to evaluate the diagnostic utility of FNAC cytology in HIV infected patients with lymphnode enlargement and to evaluate its inclusion in the protocol of six monthly surveillance of HIV infected individuals and to identify AIDS related opportunistic infections, in Indian setting.

Material and Methods

In this prospective study, fine needle aspirations were performed on 196 HIV positive individuals reporting for regular six monthly immuno-surveillance and symptomatic patients admitted in the hospital with HIV positivity from July 97 to July 2000. Detailed clinical notes were taken, patient was examined and the findings were recorded. Smears were collected, wet fixed in 95% alcohol for PAS (Periodic acid Schiff)/Grocott's stain and air dried smears were fixed in methyl alcohol for LG (Leishman Giemsa) stain and heat fixed for ZN (Ziehl-Neelsen) and Auramine-Rhodamine (AR) stain for fluorescent microscopy. All these stains were done in all the cases, irrespective of the cytological findings. If three smears were obtained LG, PAS and AR stains were done. If only two smears could be obtained, they were stained by LG and PAS and after examining the smears any of these smears were de-stained and re-stained for AR. If only one smear was obtained, it was stained initially for LG, then de-stained, re-hydrated by immersing in 0.9% saline for half an hour and postfixing in 95% alcohol for PAS and later the same smear was de-stained and re-stained for AR. When adequate material could be obtained, appropriate cultures on Lowenstein Jensen medium, Sabourad's agar and blood agar were carried out.

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Results

75% cases were asymptomatic and were referred for FNAC as lymph node enlargement was detected during the six monthly reviews and immune surveillance. Sizes of the lymphnodes varied from 0.5 cm to 3 cm. 82% of the lymph nodes sampled were less than 1 cm in size. 90 cases (46.8%) had lymph node enlargement at more than one site, the commonest combination being cervical and axillary nodes in 89 cases. Patients presenting with single site involvement showed axillary node enlargement in 74 (36.8%), cervical node in 25 (12.6%), inguinal in 3 (1.5%) and retroperitoneal location in 4 (2.3%) (Table-1). The distribution of groups of lymph nodes from different sites sampled is given in Table-2.

TABLE 1
Showing observed lymphadenopathy (n=196)

Site	Number (%)
Cervical	25 (12.6)
Axillary	74 (36.8)
Inguinal	3 (1.5)
Retro-peritoneal	4 (2.3)
Multiple sites	90* (46.8)
Total	196 (100)

* - Cervical and axillary in 89 and axillary and inguinal in 1.

TABLE 2
Lymph nodes sampled (n=196)

Site	Number (%)
Cervical	77 (39.2)
a) Jugulodigastric	6
b) Post triangle	24
c) Vertical chain	29
d) Supraclavicular	13
e) Submandibular	5
Axillary	111 (56.6)
a) Bilateral	37
b) Right	32
c) Left	42
Inguinal	4 (2.1)
Retroperitoneal	4 (2.1)

The diagnoses offered are given in Table-3. The commonest diagnosis offered was Reactive lymphadenitis in 83 (42.3%), followed by TB lymphadenitis in 67 (34.2%). Lymphomas constituted 5 cases (2.6%). 2 cases of B cell lymphoma and 1 case of immunoblastic lymphoma reported were confirmed by biopsy.

The criteria followed for the diagnosis of TB lymphadenitis were AFB positivity irrespective of cyto-morphology and / or presence of caseation necrosis with epithelioid cell granulomas. Of the 67 cases of TB lymphadenitis, 11 (16.4%) had shown only reactive pattern on cytological examination without any necrosis or granulomas. In 2 cases (2.9%), aspirate smears showed only tissue fluid or peripheral blood with very few lymphoid cells, but AFB were detected. Necrotizing lymphadenitis pattern was observed in 29(43.4%) followed by necrotizing granulomatous pattern in 20(29.9%). Granulomatous pattern was seen only in 5 (7.4%) cases. The granulomatous component comprises usually isolated epithelioid cells or small clusters of epithelioid cells (Fig-1). Well-defined granulomas were not observed. Degenerated neutrophils were seen along with necrotic debris, sometimes in

TABLE 3
Cyto-morphological diagnoses offered

Diagnosis	Number (%)
Reactive lymphadenitis	83 (42.3%)
No opinion	39 (19.9%)
a) Cervical	12 (31.5%)
b) Axillary	25 (64.2%)
c) Retroperitoneal	2 (5.1%)
Tuberculous	67 (34.2%)
a) Reactive pattern	11 (16.4%)
b) Necrotizing	29 (43.4%)
c) Necrotizing granulomatous	20 (29.9%)
d) Granulomatous	5 (7.4%)
e) Tissue fluid/Blood	2 (2.9%)
Associated with cryptococcal infection	1 (—)
Fungal	2 (1.0%)
Lymphoma/Hodgkin's	5 (2.6%)

TABLE 4
Cyto-morphological patterns observed in cases of tuberculous lymphadenitis (n=67)

Cyto-morphological pattern	Symptomatic	Asymptomatic	Total
Reactive	5	6	11
Necrotising	19	10	29
Necrotising granulomatous	13	7	20
Granulomatous	2	3	5
Tissue fluid/blood	2	-	2
Total	41 (61.2%)	26 (38.8%)	

necrotizing pattern. In 3 cases macrophages scattered and in clumps were seen with negative un-stained images inside the cytoplasm (Fig-2). These un-stained images were found to be acid fast bacilli by ZN stain (Fig-3). The details of cyto-morphological patterns observed are given in Table-4. Smear positivity was detected in 41 (61.2%) of symptomatic patients and in 26 (38.8%) cases of asymptomatic patients undergoing immune surveillance. The cyto-morphological pattern was also similar in both the groups. Smear positivity was detected even in lymphnodes of sizes as small as 0.5 cm.

In 39 cases no opinion was possible, as there were no repre-

Fig. 1 : Microphotograph showing loose aggregate of epithelioid cells intermingled with lymphocytes X 200.

Fig. 2: Microphotograph showing aggregate of macrophages with intracytoplasmic unstained images and background granular material x 1000

Fig. 3: Microphotograph showing AFB in the cytoplasm of the macrophages. ZN stain x 1000

Fig. 4: Lymphnode aspirate smear showing numerous yeast forms of cryptococcus with skeletal muscle fibres. Leishman stain x 200

sentative cells in the smears. Out of these, 64.2% were from axillary nodes, which were very small and difficult to fix. Hence, blind FNAC was performed to avoid needle stick injury to the performer. Even the blind procedure yielded cells in 72% of cases in tiny axillary nodes. A case of cryptococcal lymphadenitis asso-

ciated with TB aetiology was diagnosed. Culture of the material obtained was done in 23 cases. Of these; 21 were *Mycobacterium tuberculosis*, one isolate of *Cryptococcus neoformans* and a single case of *Rhodo torula*.

Biopsy was done to confirm cases of lymphomas and in cases of clinically unacceptable diagnosis. In 13 cases biopsy material was available for correlation. Details are given in Table-5. 4 false negatives and 2 false positives were observed. False negatives included 2 cases of tubercular aetiology and 2 cases of florid follicular hyperplasia.

TABLE 5

Showing cyto-histological correlation (n=13)

Cytomorphological diagnosis	No	Histological diagnosis		
		Reactive	TB	NHL
Reactive	6	4	2	-
NHL	4	1	-	3
Hodgkin's	1	1	-	-
No opinion	2	2	-	-

Discussion

Lymph nodes, which form the bulk of lymphoid tissue, are the major anatomic sites for establishing and propagation of HIV infection. More over lymphnodes are the filters of opportunistic pathogens. Some patients suffer progressive generalised lymphadenopathy (PGL) relatively early in the disease while others may experience varying degrees of transient lymphadenopathy. Lymphnode involvement is a common denominator of virtually all patients with HIV infection. Asymptomatic stage may extend up to 10 years or longer as reported in western literature. Symptoms are associated with onset of opportunistic infections [4]. The prolonged asymptomatic phase does not appear to be associated with Indian patients.

India is in the epicentre of HIV pandemic. Till such time a definitive therapeutic modality for AIDS is established, the strategy of management of these cases is going to be early detection and prompt treatment of opportunistic infections. Six monthly review and surveillance is essential to monitor the cases.

The opportunistic infections depend on the microbiological milieu of the individual and the community. The pattern of the disease is different in Indian scenario, compared to western literature [1,5,6,7]. Commonest infection is TB [3]. The same is reflected in our study. Sampling of even very tiny lymph nodes has yielded results in the form of smear positive TB lymphadenitis. All the smear samples should be stained routinely for AFB both by ZN and AR and for fungi by PAS/Grocott's stain, so that detectable pathogens are not missed. Llatjos et al observed three patterns of TB lymphadenitis in cytological examination

[8]. They are granulomatous lymphadenitis (GL), necrotising granulomatous lymphadenitis (NGL) and necrotising lymphadenitis (NL). Jeena et al did not find any difference in HIV infected and HIV non-infected with TB lymphadenitis in a biopsy study in children [9]. In our study we have found the fourth pattern, that is "Reactive pattern with AFB positivity" in 11(16.4%) cases. In 2.9% AFB could be demonstrated in the smears even when there was only tissue fluid in the smears. These findings were not reported earlier. The negative images of AFB in the macrophages, observed by us has also been reported by Ang GA et al [10]. In 1 case aspiration of left supraclavicular node yielded granular material showing only cryptococci with skeletal muscle fibres (Fig-4). Alfano et al, who reported 3 cases of peripheral lymph node cryptococcosis also observed similar findings [7].

By definition PGL is lymph node enlargement of over 1 cm size in two or more extra inguinal sites and the duration of lymph node enlargement should be over 3 months. Ellison E et al, reported that lymph node over 2 cm size correlated well with the fine needle aspiration diagnosis [11]. Shapiro AL et al also report similar finding [12]. Out of 196 patients we studied, only 35 (18%) had lymph nodes of size over 1 cm. Bem C in a study of 157 cases found lymph node size of 3 cm in 36%, 2 cm in 24% and 1 cm in only 6% nodes [13]. In our study lymphadenopathy was picked up by the clinicians during six monthly surveillance well before the symptoms appeared. Hence, 82% of the nodes sampled are of size 1 cm or smaller. 26 of 147(17.7%) asymptomatic patients were diagnosed as smear positive TB lymphadenitis, though the lymph nodes were 1 cm or less in size. Thus opportunistic infections were picked up early by surveillance of cases.

In a large western study 37% of the aspirates were benign / reactive, 13% suspicious of malignancy, 14% specific infections with stainable organisms, 16% inflammatory and 20% were reported as inconclusive [11]. Bottles et al reported hyperplasia in 50%, NHL in 20%, Kaposi's sarcoma in 10% and a case each of Hodgkins's disease, giant cell carcinoma, nasopharyngeal carcinoma and squamous cell carcinoma [14]. They have detected only 17% of mycobacterial disease. Our study had shown more of specific pathogens (35.7%) and less of malignancies (2.9%) (Table 3. Majority (64.2%) of the inconclusive diagnoses were from axillary nodes, where satisfactory sampling could not be done.

Aspirate in one case was suggestive of Hodgkin's

disease whereas biopsy revealed findings of Kimura's disease. Repeat biopsy of the same patient after a month from different site was done as new nodes appeared, which showed folliculolysis with AFB positivity. Association of HIV and Kimura's disease has not been reported in literature. False positivity was noticed in 2 cases. These include one case of dermatopathic lymphadenopathy reported as suspected lymphoma and a case of Kimura's disease as Hodgkin's disease. False negativity was observed in 2 cases of tuberculous lymphadenitis, reported as reactive by FNAC and in 2 cases florid follicular hyperplasia was inconclusive in aspiration study. Bottles et al report no false positivity and 5 cases of false negativity in a study of 121 cases [14].

Correlation of immune status could have been possible by doing CD4 and CD8 counts [15]. FNAC is being tried to evaluate the disease status and clinical staging by flow cytometric analysis of aspirated cells [16] and for monitoring of response to anti-retroviral therapy [17]. Opportunistic viral agents and toxoplasmosis could have been missed by the cyto-morphological study and the limited special stains used in the study. Immuno-fluorescence kits for *Human papilloma virus*, *Cytomegalovirus*, *Toxoplasma* etc, could have picked up these infectious agents as well in the smears.

FNAC is an excellent tool for evaluation of lymphadenopathy in HIV positive patients. FNAC should be performed even when the lymph nodes are less than 1 cm size. Axillary lymph nodes pose problem for the performer of the procedure, but blind FNAC without fixing the node should be attempted as this also provides useful material. TB is the major opportunistic infection, which could be detected in both the asymptomatic and symptomatic individuals and even with tiny nodes. AFB and other specific pathogens should be looked for even when the cytological picture is suggestive of reactive pattern and cellularity is scanty. Presence of intra-cytoplasmic negative images should raise suspicion and ZN stain should be done to pick up AFB. FNAC is useful for detection of mycoses and neoplasia in lymph nodes of HIV infected patients. All suspected neoplasia should be biopsied to avoid false positivity. Infection by *Rhodo torula* in HIV positive patient is reported for the first time. Association of Kimura's disease and HIV is reported for the first time.

CD4, CD8 counts done along with needle aspiration will give better clinico-pathological correlation, as immune status vis-a-vis the opportunistic infections in Indian setting can be assessed. Immuno-fluorescence

for viral antigens and *Toxoplasma*, if done, could pick up these infectious agents better on the smears.

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