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RELATIONSHIP OF CD4 COUNT WITH FNAC FINDINGS OF LYMPH NODE IN HIV PATIENTS WITH LYMPHADENOPATHY

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Conflicts of Interest: Nil

Abstract:
As the HIV virus primarily infects the lymphocytes, lymph nodes are commonly involved during all stages of infection and are one of the earliest signs in HIV infected patients. The most common conditions affecting the lymph nodes in HIV positive patients are: reactive changes, including progressive generalized lymphadenopathy (PGL); opportunistic infections with Mycobacterium tuberculosis, Mycobacterium avium intracellulare, Histoplasma capsulatum and Cryptococcus neoformans and malignant conditions such as non-Hodgkin's and Hodgkin's lymphomas and Kaposi's sarcoma. The present study was performed to evaluate the role of FNAC as a cytological investigative tool in the diagnosis of various lesions in HIV lymphadenopathy[9-20] and correlates the cytological findings with CD4 count of the patient. The study reveals the correlation between absolute CD4 count and FNAC findings .The median value CD4 count was 256 cells/cumm in case of reactive hyperplasia and 161 cells/cumm in Tuberculous lymphadenitis.

Keywords: HIV, Lymphadenopathy, cytology, CD4 Count

1. Introduction

The scourge of AIDS (Acquired Immune Deficiency Syndrome) as appropriately described by Colin Powell, as he told the UN more than a year after 9/11: "AIDS is more devastating than any terrorist attack, any conflict or any weapon of mass destruction. As the HIV virus primarily infects the lymphocytes, lymph nodes are commonly involved during all stages of infection and are one of the earliest signs in HIV infected patients.[1, 2] Lymphadenopathy may also be a manifestation of opportunistic infections, lymphoid malignancy developing in an immunodeficient individual[5]. The most common conditions affecting the lymph nodes in HIV positive patients are: reactive changes, including progressive generalized lymphadenopathy (PGL); opportunistic infections with Mycobacterium tuberculosis, Mycobacterium avium intracellulare, Histoplasma capsulatum and Cryptococcus neoformans and malignant conditions such as non-Hodgkin's and Hodgkin's lymphomas and Kaposi's sarcoma.[2, 3,4] FNAC has become the primary investigative procedure for mass lesions on HIV-positive patients, particularly in the assessment of lymphadenopathy[5-9].The present study was performed to evaluate the role of FNAC as a cytological investigative tool in the diagnosis of various lesions in HIV lymphadenopathy[9-20] and correlates the cytological findings with CD4 count of the patient.[21-24]

AIMS AND OBJECTIVES

Aim of the study was to study patterns of lymphadenopathy in HIV positive cases by Fine needle aspiration cytology (FNAC) also to Study relationship between Lymph node cytology and CD4 count in HIV positive cases with lymphadenopathy.

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MATERIAL AND METHODS

The present study was undertaken in the Department of Pathology, from January 2017 to December 2018. During this period 62 fine needle aspirates were obtained from 62 Known HIV positive patients who presented with lymphadenopathy. All the patients were studied prospectively with informed consent of the patient. FNAC was performed in Surgical Minor Operation Theatre or in the respective wards. Lymph nodes other than inguinal lymph node were aspirated. Each lesion was aspirated either once or twice and specimen adequacy was checked at the time of aspiration. All the smears were stained by Papanicolaou stain and Ziehl Neelson stain (Z N stain) for acid fast bacilli (AFB). The specimen for histopathology examinations were obtained by surgical biopsy or surgical excision. Paraffin sections were stained with Hematoxylin & Eosin stain in as many cases as possible. Special stain were employed as and when necessary. Cytopathologic diagnosis was compared with histopathologic diagnoses whenever available.

The smears prepared from Fine Needle aspiration from the lymph node of the patients were stained and studied. All the smears were stained with Papanicolau stain and Ziehl Neelson stain. Pattern of smears in all the cases were viewed in background of clinical details. All smears screened under low power and high power magnification of microscope and classified into four categories:

1. Reactive lymphadenopathy.
2. Tuberculous lymphadenopathy.
3. Suppurative Lymphadenopathy. (Inflammatory smear)
4. Positive for malignant (Lymphoma/ other) cells.
5. Unsatisfactory for opinion.

CD4 count of each patient was done with the help of flow cytometer (The BD FACS Calibur™ system) by Becton Dickinson.

OBSERVATIONS AND RESULTS

Table 1: Age wise distribution of cases

Age group	No. of patients	% of patients
<20	1	1.61
21-30	12	19.35
31-40	34	54.83
>40	15	24.19
Total	62	100

In our study , we have included patients of all age groups and we subdivided this range into groups, < 20 years, 21-30 years ,31-40 years and >40 years. We found 54.83% of the patients belonged to age group of 31-40 years.The sex distribution of cases for FNA diagnosis revealed a male predominance. There were 46 (74.19%) males and 16 (25.8%) females out of the total 62 patients.

Table 2: Sexwise Distribution of Study Cases

Sex	No. of patients	% of patients
Female	16	25.8
Male	46	74.19
Total	62	100

Out of 62 patients 11(17.74%) aspirates were from axillary group of lymph node and 51 (82.25%) aspirates were from cervical group of lymph node.Total 62 HIV positive cases with Lymphadenopathy were submitted for FNAC. Of these 24 cases were typed as suppurative. 10 cases as tuberculous lymphadenopathy, 23 cases as reactive, 2 as neoplastic and 2 as unsatisfactory smears.

Table 3: FNAC SMEAR Patterns of Lymph Nodes According to Pathology.

FNAC findings	Patients	%
Suppurative lymphadenopathy	24	38.7
Reactive Hyperplasia	23	37.09
Tuberculous lymphadenopathy	10	16.12
Lymphoma	2	3.22
Other (unsatisfactory)	3	4.83
TOTAL	62	100

Suppurative Smear Pattern Out of the total 60 cases 24 (38.7%) of our patients had smear pattern consistent with suppurative lymphadenopathy.

In all these cases ZN stain was negative for AFB. All the Fine needle aspirates of suppurative lymphadenopathy smears revealed degenerated neutrophils and lymphocytes and the aspirate was purulent.(Fig. 1) One case with suppurative pattern was AFB positive and this was then included as tuberculous lymphadenopathy (Fig.2). 8 out of 24 smears of the suppurative lymphadenopathy also show degenerated neutrophils and necrotic background along with plasmacytoid cells, few eosinophils and many macrophages.

Table 4:

Pattern	No. of cases
Suppurative pattern	16
Suppurative pattern with plasmacytoid cells, eosinophils and macrophages	8
Suppurative pattern with AFB positive (Included in TB)	1

Reactive smear pattern out of total 60 cases 23(37.09 %) patients had lymph node aspirate consistent with reactive changes. The diagnosis of reactive changes was based on finding of a heterogeneous population of cells in the aspirate. These cells included a spectrum of small and large lymphocytes some of which were in mitosis. Fragments of reactive follicular centre cells, including tingible body macrophages, syncytia of dendritic reticulin cells, and some plasmacytoid cells were also seen (Fig. No 3 and 4).All the cases revealing reactive pattern were negative for AFB by ZN stain.

Tuberculous Smear Pattern

Table 5: Tuberculous Smear Pattern In Study Cases

Pattern	No. of cases
Caseation and few epitheloid cells AFB positive	4
Caseation and epitheloid cells and langhans giant cells AFB positive	2
Suppurative pattern AFB positive	1
Epitheloid cell granuloma and Langhans giant cells AFB negative	1
Caseation only AFB positive	2

The FNA material obtained from 10 (16.12%) of the total 62 cases had smear pattern following tuberculous lymphadenopathy revealed caseation necrosis and epithelioid granulomata which was identical in appearance with those seen in HIV negative patients. The granulomata were composed of syncytial aggregates of oval or fusiform epithelioid histiocytes with "blunt ended" and sometimes bent or curved nuclei. Langhans' type giant cells were occasionally seen. Definitive diagnosis of tuberculosis was made only when acid fast bacilli were demonstrated on Ziehl-Neelsen stain. In the absence of acid fast bacilli or other organisms, the presence of caseation necrosis or granulomas in lymph node aspirates in HIV positive subjects was reported as consistent with tuberculosis unless proved otherwise. Of all the cases revealing tuberculous lymphadenopathy 4 cases had caseation and only few epitheloid cells (Fig 5 and 6) 2 cases revealed caseation along with epitheloid cells and Langhans type of giant cells. All these cases were positive for AFB (Fig No.9 and 10) 2 cases of all the cases of tuberculosis revealed only caseation on smear pattern. Both the cases were positive for ZN stain for AFB. (Fig No.7 and 8) Only one case revealed well formed epitheloid cell granuloma and Langhans giant cell. Though the ZN stain in this case was negative for AFB the pattern was taken as consistent with tuberculous lymphadenopathy. (Fig.No.11) One case which had suppurative lymphadenopathy on smear pattern but positive for ZN stain was included in tuberculous lymphadenopathy.

Neoplastic smear pattern

The neoplastic lesion reported in the 2 (3.22 %) patients in the present study was non- Hodgkin's lymphoma. Smear revealed monotonous cells population of small to medium sized lymphoid cells slightly larger than lymphocytes. Some of nuclei were cleaved and had a dispersed chromatin. Further typing was not possible. Lymph node biopsy was performed in both the cases .Histopathological examination revealed lymphoma. (Fig No.13, 14) One case reported that of diffuse large B cell lymphoma with CD20,CD3 positivity and CD30 was negative Other case was Hodgkin's lymphoma with CD3 positive CD20 positive CD30 positive in large cells.

Unsatisfactory smear pattern

2 (4.83%) cases revealed mainly haemorrhage and very scanty cellularity and were considered as unsatisfactory for reporting. These three cases were not considered for correlation with CD4 count and hematologic profile.

Out of 60 patients 24(38.7%) patients had suppurative lymphadenopathy. In these patients' absolute CD4 count ranges from 60 to 1048 cells/cumm. The median of CD4 counts for these patients was 478. 23 (37.09%) patients had reactive lymphadenopathy. In these patients absolute CD4 count ranges from 24 to 478 cells/cumm.The medianCD4 count for these patients was 156 cells/cumm Ten (16.12%) patients were found to have tuberculosis. They had their CD4 count in the range 60 to 323 cells /cumm. The median CD4 count was 161cells/cumm.Two (3.22%) patients of lymphoma had median CD4 count of 24 cells /cumm.

Table 6: Showing Corelation of Cd4 Range and the Cytological Diagnosis after Deleting 2 Cases Which were Unsatisfactory for Reporting

CD4 range (cells /cumm)	Median value of CD4 count (cells /cumm)	Cytological diagnosis by FNAC	No. tested	No. positive
60-1048	478	Suppurative lymphadenopathy	60	24
24-478	256	Reactive lymphadenopathy	60	23
60-323	161	Tuberculous lymphadenopathy	60	10
24	24	Lymphoma	60	2

The total (24) cases of suppurative lymphadenopathy 16 cases had pattern typical of suppurative lesion i.e. degenerated neutrophils and lymphocytes along with necrotic background.with average CD4 count of 405 cells/cumm. Of all these 16 cases with suppurative lymphadenopathy, 12 patients had a CD4 count above 200cells/cumm and remaining 4 cases had CD4 count below 200cells/cumm 8 cases of suppurative lymphadenopathy had smear pattern of suppurative along with plasmacytoid cells and eosinophils and macrophages. The average CD4 count for these cases was 310 cells/cumm.Out of these 8 cases of suppurative lymphadenopathy along with plasmacytoid cells and eosinophils and macrophages , 5 cases had CD4 count above 200 cells/cumm.and remaining 3 had CD4 count below 200 cells/cumm.One case with smear pattern of suppurative but positive for AFB stain had CD4 count 215 cells/cumm.

REACTIVE SMEAR PATTERN

Out of total 60cases with satisfactory smears, 23(37.09 %) patients had lymph node aspirate consistent with reactive changes. The diagnosis of reactive changes was based on finding of a heterogenous population of cells in the aspirate. These cells included a spectrum of small and large lymphocytes some of which were in mitosis. Fragments of reactive follicular centre

cells, including tingible body macrophages, syncytia of dendritic reticulin cells, and some plasmacytoid cells were also seen.(fig.)..All the cases revealing reactive pattern were negative for AFB by ZN stain. Out of total 23 cases revealing reactive smear pattern, 15 cases had CD4 count above 200 cells/cumm and the remaining 8 cases had CD4 count below 200 cells/cumm.

Table 7:

	Suppurative	Reactive	Tuberculous	Lymphoma
CD4>200 cells/cumm	**17(70.83%)**	**15(65.21%)**	**5(50%)**	**0**
Cd4 <200 cells/cumm	**7(29.16%)**	**8(34.78%)**	**5(50%)**	**2(100%)**

Of the total 10 cases of tuberculous lymphadenopathy 4 cases had caseation and only few epitheloid cells on smear AFB was positive in these cases .The median CD4 count for these cases was 286 cells/cumm.Out of these 4 cases 3 had CD4 count below 200 cells/cumm.and remaining 1 had CD4 count above 200 cells /cumm.Two cases of tuberculos lymphadenopathy had caseation epitheloid cells langhans giant cells and were AFB positive. They had average CD4 count208 cells/cumm.One case had CD4 count above and one case had CD4 count below 200 cells/cumm.One case had suppurative pattern but positive for AFB stain had CD4 count 215 cells/cumm. One case had smear showing epitheloid cells granuloma and Langhans giant cells. Though AFB stain was negative in this case it was considered to consistent with tuberculous lymphadenoapthy. The CD4 count of this case was 312 cells/cumm.Two cases had only caseation on smear patterns but AFB stain was positive .The median CD4 count for these cases was 126 cells/cumm. Both cases had Cd4 count below 200 cells/cumm. Of all the10 cases with tuberculous lymphadenopathy 9 had AFB positivity. Remaining One case which revealed epitheloid granuloma with Langhans giant cell and scanty caseation was negative for AFB. Of the 9 AFB positive cases 7 had CD4 count below 200 cells/cumm. And 2 cases had CD4 count above 200 cells/cumm.The remaining AFB negative case labled as consistent with tuberculous lymphadenopathy had CD4 count of 447 cells/ cumm.

TUBERCULOUS SMEAR PATTERN

Table 10: Tuberculous Smear Pattern and Correlation with Cd4 Count

Pattern	No. of cases	Average CD4 count	CD4 count above 200 cells/cumm	CD4 count below 200 cells/cumm
Caseation and few epitheloid cells AFB positive	4	286	1(25%)	3(75%)
Caseation and epitheloid cells and langhans giant cells AFB positive	2	208	1(50%)	1(50%)
Suppurative pattern AFB positive	1	215	1(100%)	0
Epitheloid cell granuloma and Langhans giant cells AFB negative	1	312	0	1(100%)
Caseation only AFB positive	2	126	2(100%)	0

Strigle SM. Et al studied the patients infected with the human immunodeficiency virus (HIV). During a two year period, 396 aspirates were performed on 362 HIV-infected patients within the LAC-USC Medical Center. Significant pathologic processes identified in these patients by means of FNA included reactive lymphoid proliferations (35%), abnormal lymphoid proliferations (12%), infections (12.5%), cystic (5.5%) and inflammatory processes (5%), nonlymphoid malignancies (4%), and salivary gland pathology (1%).

Reid AJ et al[19] (1998) Sixty-five FNA cytology procedures were performed on lymph nodes in 52 HIV+ patients. Cervical lymph nodes were the commonest site of FNA cytology investigation (54%). The diagnoses were persistent generalized lymphadenopathy (38%), infection (17%), and malignancy (11%). Diagnosis could not be rendered in 25% of FNA cytology due to inadequate sampling. Of those with infection, mycobacterial disease was the commonest cause (91%), the diagnosis of which was enhanced by concurrent microbiological examination. In a similar study Jayaram G et al.[20] (2000) assessed the role of fine needle aspiration cytology (FNAC) in lymphadenopathy in human immunodeficiency virus-infected individuals (HIVII). Thirty-nine HIVII presenting with lymphadenopathy at University Hospital, Kuala Lumpur, were studied. In 21 cases, acid-fast bacilli (AFB) were demonstrated in the cytologic smears, enabling a diagnosis of mycobacterial lymphadenitis. Among the Indian reports Uma Nahar Saikia et al.[12] (2001) evaluated the role of fine needle aspiration biopsy (FNAB) material in 25 HIV-positive cases with lymphadenopathy. All these patients were heterosexual, and none had a history of drug abuse. The most common FNAB diagnosis was reactive lymphoid hyperplasia, followed by tuberculosis. Satyanarayana et al[14] (2002) Fine needle aspiration cytology of 196 HIV positive patients was studied during six monthly review Commonest opportunistic infection noticed was tuberculosis (TB) in 34.2 percent. Cyto-morphologically reactive pattern with Acid fast bacilli (AFB) positivity was observed in 16.4 percent of TB cases. Another similar study by Shenoy R. et al[16] (2002) at Manglore evaluate the usefulness of fine needle aspiration (FNA) study of lymph nodes in HIV-positive patients. They found Tuberculosis in nearly half (48%) the cases, followed by HIV lymphadenitis (36%), lymphoma (10%), suppurative lymphadenitis (2%), Mycobacterium avium-intracellulare lymphadenitis (2%) and metastases (2%), in descending order of their frequency contracting the infection.

In a study by Sujata Nayak et al. (2003) Fine-needle aspiration cytology (FNAC) of 32 HIV-positive cases presenting with lymphadenopathy was performed to evaluate its role in this group of patients. The results were tuberculous lymphadenopathy (15) reactive lymphadenopathy (10), acute lymphadenitis /abscess (5), and suspected malignancy (2). In a study by Vanisri H. R et al.[15] (2008) Thirty-six human immunodeficiency virus (HIV)-positive patients with lymphadenopathy were subjected to fine-needle aspiration cytology (FNAC) over a period of 2 years.

The maximum number of cases had tuberculosis (58.3%) followed by reactive lymphadenitis (36.1%), non-Hodgkin's lymphoma (2.7%) and acute suppurative lymphadenitis (2.7%). Shobhana et al.[21](2002) study at tropical school of medicine, kolkata Study revealed only 3 types of pathology in lymphadenopathy cases by FNAC. Out of 54 subjects with lymphadenopathy , FNAC showed features of reactive hyperplasia in 30 cases (55.5%) evidence of tuberculous lymphadenitis and NHL was found in 22(41%) and 2(3.7% cases respectively . The study also reavled correlation between absolute CD4 and FNAC findings . the median value of CD4 count was 672 and 212 cells/ul in case of reactive hyperplasia and TB lymphadenitis respectively. It is generally considered that the CD4 count decrease is much more evident in lymphoma where the decrease may fall below 100 cells /ul and observation of the study corroborates well with description in other literature study by Khiste J et al and Rao JS et al[22,23]. There is no doubt that biopsy is a better diagnostic tool , but FNAC can serve as an alternative method and may be practiced for diagnosis of opportunistic infections in HIV/AIDS i.e. tuberculosis

.histoplasmosis , toxoplasmosis ,and malignant conditions like Kaposi' sarcoma and lymphomas etc. Apart from cytological examination, culture of the aspirated material in appropriate media may increase the diagnostic efficiency.

CONCLUSIONS

The study reveals the correlation between absolute CD4 count and FNAC findings .The median value CD4 count was 256 cells/cumm in case of reactive hyperplasia and 161 cells/cumm in Tuberculous lymphadenitis

Search for Acid Fast Bacilli in the FNAC material revealed one case with Suppurative smear pattern was found to be AFB positive. This indicates that search for AFB is useful to identify tuberculous lymphadenitis as this infection responds well to antituberculous drug regimen even with the background of HIV positivity, as compared to other opportunistic infections.

The search for AFB is useful to identify tuberculous lymphadenitis as this is a treatable infection even with the background of HIV positivity and hence the search for AFB is recommended in all cases of HIV with lymphadenopathy.

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FIGURE DETAILS

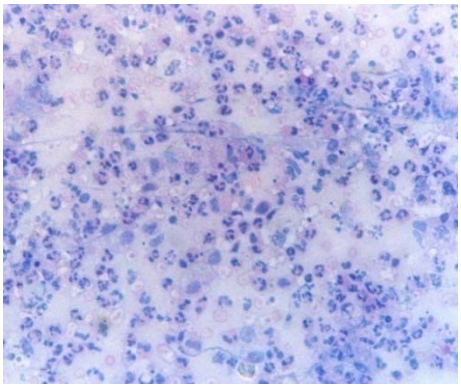

Figure 1: Suppurative smear pattern showing degenerated neutrophils and lymphocytes. (PAP stain X 10 x)

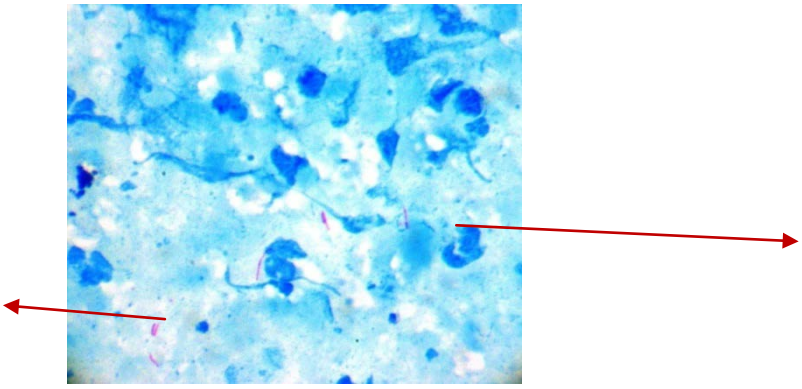

Figure 2: One case of Suppurative smear pattern revealed AFB positivity (ZN Stain x 100x i.e. oil immersion)

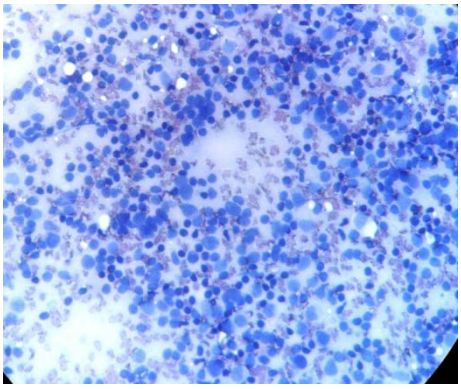

Figure 3: Reactive smear pattern showing heterogenous population of cells. (Pap stain X 10x)

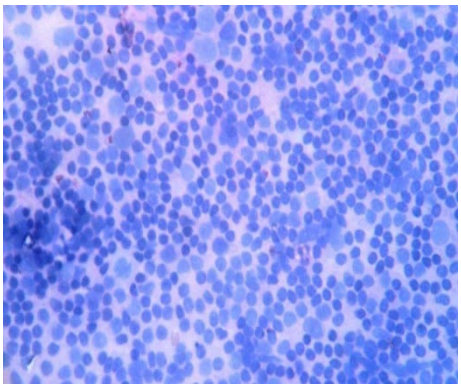

Figure 4: Reactive smear pattern showing follicular centre cells, including tingible body macrophages, syncytia of dendritic reticulin cells(pap X40)

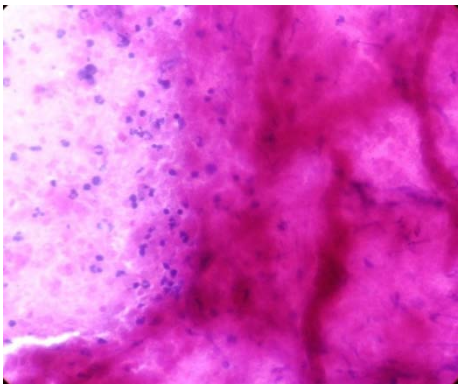

Figure 5: Tuberculous smear pattern showing only Caseation and few epitheloid cells (PAPX40)

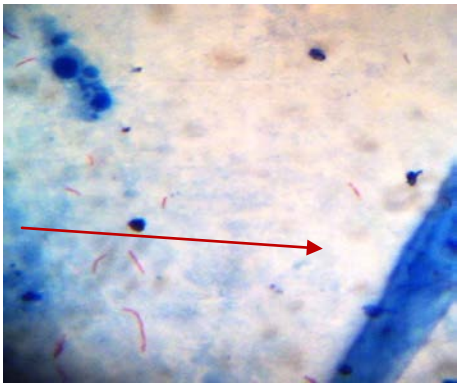

Figure 6: Above case AFB positive (ZN stain X100 i.e. oil immersion)

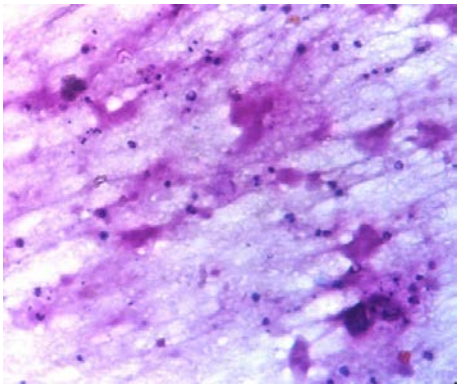

Figure 7: Tuberculous smear pattern showing Caseation only. (PAP X10)

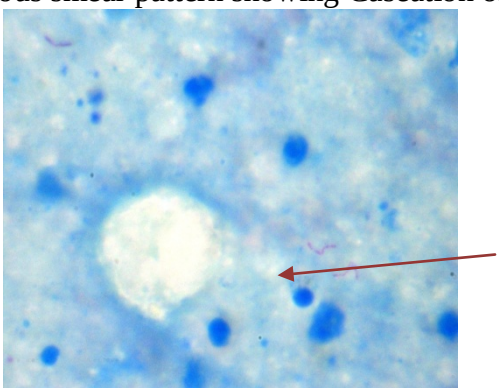

Figure 8: Above case showing AFB positivity (ZN stainX100)

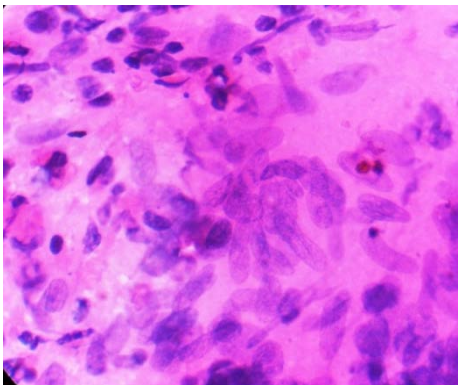

Figure 9: Tuberculous smear pattern with well formed epitheloid cell granuloma(PAP x40)

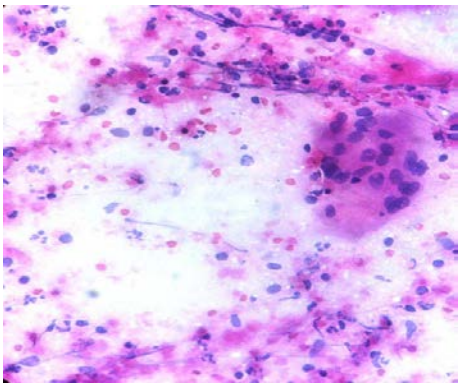

Figure 10: Tuberculous smear pattern: Caseation and epitheloid cells and Langhans giant cells (PAPX40)

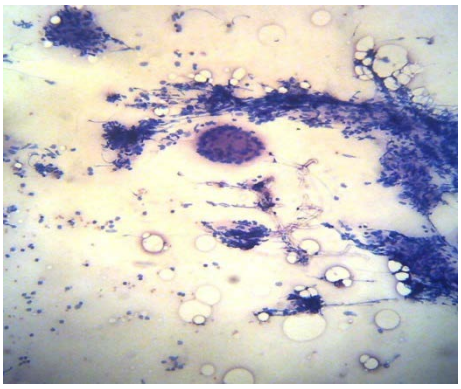

Figure11: Consistent with tuberculosis: Epitheloid cell granuloma and Langhans giant cells (PAP X 40)
This case was found to be AFB negative.

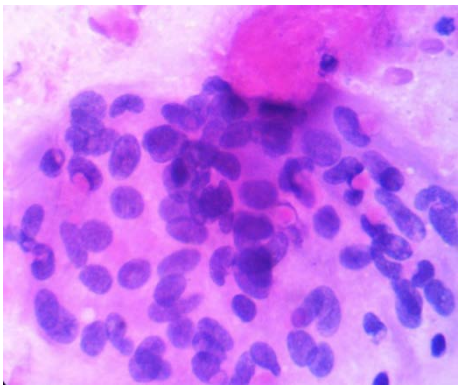

Figure 12: Tuberculous smear pattern with ill formed granuloma (PAP X 40)

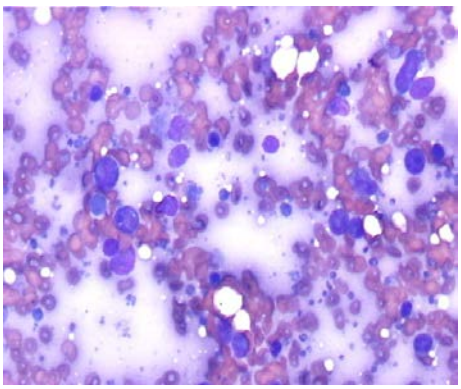

Figure 13: Lymphoma smear pattern Cytology(PAP x 40)

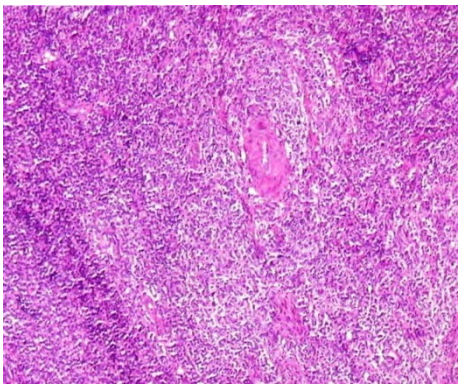

Figure 14: Histopathology of the above case: Non Hodgkin's lymphoma (H&E X20)

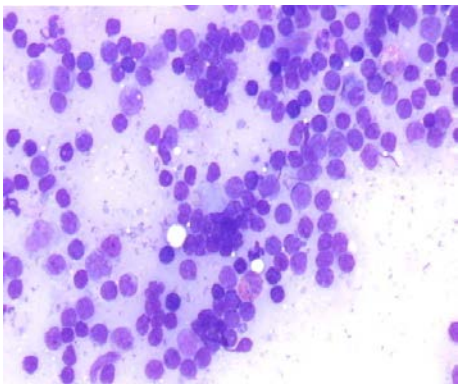

Figure 18: Lymphoma smear pattern (PAPx40)