



ORIGINAL RESEARCH PAPER

CLINICOPATHOLOGICAL STUDY OF CORRELATION BETWEEN FNAC SMEAR , ZN STAINED SMEAR & HIV STATUS IN CLINICALLY SUSPECTED CASES OF LYMPH NODE TUBERCULOSIS IN URBAN EASTERN INDIA

Pathology

KEY WORDS:tuberculous Lymphadenitis (TBL), Fine Needle Aspiration Cytology (FNAC), Acid Fast Bacilli (AFB) , Ziehl Neelsen (ZN) .

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ABSTRACT

BACKGROUND: Tuberculous lymphadenitis (TBL) is the commonest form of extra pulmonary tuberculosis. Fine Needle Aspiration Cytology (FNAC) is a simple outpatient diagnostic procedure used in the diagnosis of TBL and all stages of Human Immunodeficiency Virus (HIV) infection are associated with increased risk of tuberculosis (TB).
AIM & OBJECTIVE: To correlate different cytomorphologies of FNAC smears and presence or absence of Acid fast bacilli (AFB) in the Ziehl Neelsen (ZN) stained smear prepared from the same aspirate. Association of HIV infection are also to be evaluated.
METHOD: An observational cross-sectional study was conducted at department of pathology in collaboration with department of pulmonary medicine, Medical College & Hospital, Kolkata from January 2018 and June 2019. It included 305 cases with enlarged palpable lymph nodes. FNAC done; aspirated smears were stained with papanicolaou, Leishman-Giemsa and ZN stains. HIV status were also noted.
RESULTS: 251 aspirates were reported on cytomorphology as suggestive of TBL with 117 (47.01 %) AFB positivity in ZN stained smears. Cervical lymph node were most commonly involved site (86.84 %) and females (63.75%) were affected more than males (36.25%). Most cases of AFB positive smears were associated with caseation necrosis only (71.8 %). 17 (6.77 %) out of 251 cases were co infected with HIV.
CONCLUSION: Special stains should be done in all suspected cases of TB lymphadenitis and most common cytomorphology associated with AFB positivity is caseation necrosis without granuloma. Also it can be concluded that TB/HIV co infection is not predominant in India.

INTRODUCTION

Tuberculosis (TB) is a serious chronic pulmonary and systemic disease caused most often by Mycobacterium tuberculosis. [1] It is associated with high levels of morbidity and mortality, especially in developing countries. Approximately, one-third of the world's population is currently infected with the TB bacillus. [2] Pulmonary tuberculosis is the most common involvement whereas tuberculous lymphadenopathy is the commonest form of extra pulmonary TB. [3]

Today there are many methods available for the diagnosis of TB Lymphadenitis, such as Fine needle aspiration cytology (FNAC), Biopsy, Ziehl Neelsen (ZN) staining, culture by Lowenstein -Jensen (LJ) media, culture by Radiometric BACTEC method, Polymerase Chain reaction (PCR) etc. [4] Because of early availability of results, simplicity, minimal trauma and complication, the aspiration cytology is now considered as a valuable diagnostic aid. [5] Developing countries, especially those in South Asian region, are having a major burden of tuberculosis. [6] In regions where TB is very common, the morphological findings of granulomatous inflammation is strongly suggestive of tuberculosis. Since epithelioid granulomas, caseation necrosis, giant cells and AFB positivity are strongly suggestive of TB, so in these countries excision biopsy can be avoided and antituberculous treatment can be given straightway. [7]

Excision is not free of complication and other methods are time consuming and expensive, thus they can delay treatment. Above findings conclude that FNAC with special stains can adequately help the physician to start treatment.

Infection with HIV significantly increases the risk of developing active tuberculosis (TB). An individual infected with HIV is 25 times more vulnerable to TB in contrast to non-infected individuals, and the risk of death in patients who are co infected with HIV and Koch's bacillus is twice as higher

than in an HIV-seropositive individual without TB. [8] Also the pool of HIV infected persons in Asia is large and expanding. [11] So the HIV status of the patients are also included in this study.

OBJECTIVE

The present study aimed at describing the different cytomorphological features of FNAC smears aspirated from clinically suspected TB lymphadenitis cases and their correlation with presence or absence of Acid - fast bacilli in the ZN stained smear prepared from the same aspirated material. Coinfection of HIV with tuberculosis are also evaluated in this population.

MATERIALS AND METHODS

This institution based cross-sectional observational study was commenced after approval from institutional ethics committee. Patients of all ages and both genders attending the Pulmonary Medicine outpatient department of our hospital, between the time period of January 2018 and June 2019 with well palpable and enlarged peripheral lymph nodes & clinical features suggestive of tuberculous lymphadenitis were included. Their clinical history was obtained and detailed physical examination performed. Routine investigations,

X-ray and appropriate serological markers were tested to corroborate clinical suspicion. Patients who do not provide informed consent were excluded. Next, the cases with a clinical diagnosis of tuberculous lymphadenitis were subjected to FNAC. Varying sites of lymphadenopathy i.e. cervical, axillary, inguinal were aspirated using 22 gauge needle attached to 10ml disposable syringe under strict aseptic conditions. During each pass the needle was moved throughout the lesion several times while aspirating. In each case part of the aspirate was used for preparing three smears, one for papanicolaou stain, which was fixed immediately in cytofix containing 95% ethyl alcohol and one for Leishman-Giemsa stain and one for Ziehl-Neelsen (ZN) stain. Smears for

later two staining were kept for air drying. The gross appearance of aspirate was described as caseous when it was cheese like or yellow white aspirate, pus when it was greenish yellow or yellowish aspirate and blood mixed when material was hemorrhagic. The stained smears were examined under light microscope and oil immersion lens are used to examine ZN stained smears. The Acid Fast Bacilli positivity was labeled after finding red or pink rod-shaped bacteria with beaded appearance. Observations are noted down and analyzed. HIV status of the patients are collected.

RESULTS

Among 305 cases, 251(82.3%) aspirates were reported on cytomorphology as suggestive of tuberculous lymphadenitis; 40(13.2%) cases had reactive lymphadenitis, 7(2.3%) cases had suppurative lymphadenitis, 4(1.3%) cases had metastatic deposit and 2(0.6%) patients had cytomorphology suggestive of lymphoma.

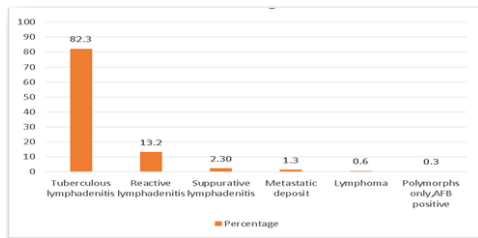


Fig: 1 Showing Distribution of various Lymph node lesions (t=305)

Among 251 patients suggestive of TBL, 157 (62.55%) were female and 94(37.45%) were male. The minimum and maximum age of the patients were subsequently 2yrs and 85 years with mean 28.61 & standard deviation 14.73. Majority of the patients (59.37%) belong to the age group 11-30 years (28.29% in the age group 11-20 years and 31.08% in the age group 21-30 years). Combined age & gender distribution of patients are depicted in Table 1 & Figure 2.

Table 1: Combined Age & Gender Distribution of Patients (n=251)

Age Group	Female	Male	Total	Percentage
1-10	6	8	14	5.58%
11-20	50	21	71	28.29%
21-30	49	29	78	31.08%
31-40	21	17	38	15.14%
41-50	13	13	26	10.36%
51-60	10	5	15	5.98%
61-70	6	1	7	2.79%
71-80	1	0	1	0.40%
81-90	1	0	1	0.40%
	62.55%	37.45%		100%
Grand Total	157	94	251	

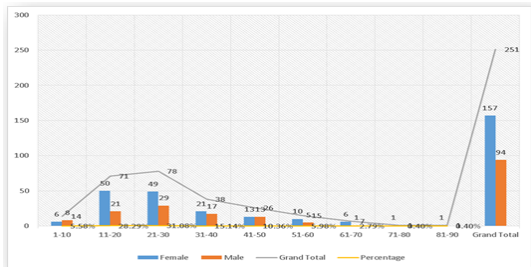


Fig: 2 Showing Combined Age & Gender Distribution of Patients (n=251)

Among 251 cases, 181 cases (72.11%) had cervical lymph node involvement. Supraclavicular and axillary lymph nodes were involved in 30(11.95%) & 22(8.76%) cases respectively.

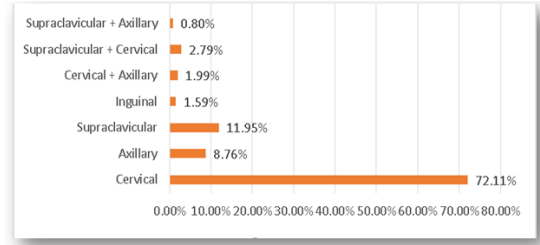


Fig-3 Distribution of site of lymph nodes (n=251)

Among them 85(33.86%) patients presented with single lymph nodes and 166(66.14%) with multiple lymph node enlargement. The duration of lymph node enlargement varied from 10 days to 2 years.

Table 2: Frequency of Involved Number of Lymph node (n=251)

No of Lymph node	No of Cases	percentage
Multiple	166	66.14%
Single	85	33.86%
Grand Total	251	100%

Among 251 cases, 146 (58.17%) patients had history of fever. History of cough and weight loss were present in 66(26.29%) & 25(9.96%) patients while 75(29.88%) patients had no symptoms other than lymphadenopathy. 76(30%) patients had contact history of TB and 35(14%) cases had previous history of TB. Sixteen (6%) patients were on ATD at the time of diagnosis. Relevant history & associated symptoms are depicted in table-3&4.

Table 3: Distribution of Associated symptoms (n=251)

Associated symptom	No of cases	Percentage
Fever	146	58.17%
Weight loss	25	9.96%
Cough	66	26.29%
No symptom	75	29.88%
Sinus formation	7	2.79%
Ear discharge	1	0.40%
hemoptysis	4	1.59%
Oral thrush	1	0.40%
pleural effusion	7	2.79%
pneumonitis	1	0.40%
Respiratory distress	2	0.80%
Ulceration	1	0.40%

Table 4: Distribution of Relevant History (n=251)

Case History	No of Cases with Positive History	Percentage
contact history of TB	76	30%
Previous history of TB	35	14%
On ATD	16	6%
Count of Cases	251	100%

The gross appearances of aspirated material were classified into caseous, purulent, blood mixed and serous material. Presence of acid fast bacilli was compared with gross appearance. It was found that AFB seen mostly in the smears prepared from purulent material (72.1%) followed by caseous material (58.82%). the distribution is depicted in table 5

Table 5: Distribution of Gross appearance of aspirate with AFB status (n=251)

Aspirate	AFB Present	AFB Absent	Grand Total
Caseous	40(58.82%)	28(41.18%)	68(27.09%)
Purulent	31(72.1%)	12(27.9%)	43(17.13%)
Blood mixed	43(31.61%)	93(68.38%)	136(54.18%)
Serous	2(50%)	2(50%)	4(1.59%)

Based on cytological (FNAC) findings, 251 cases could be diagnosed as cases suggestive of tuberculous lymphadenitis. Out of them, 116(46.22%) demonstrated acid fast bacilli on respective Z-N stained smears. These cases were subdivided into three groups on the basis of presence of necrosis and epithelioid cell granuloma and a single case had polymorphs only but was AFB positive on ZN stained smear. Most commonly observed cytomorphology is granuloma without necrosis (40.23%) followed by granuloma with necrosis (34.26%) and necrosis without granuloma (25.49%).

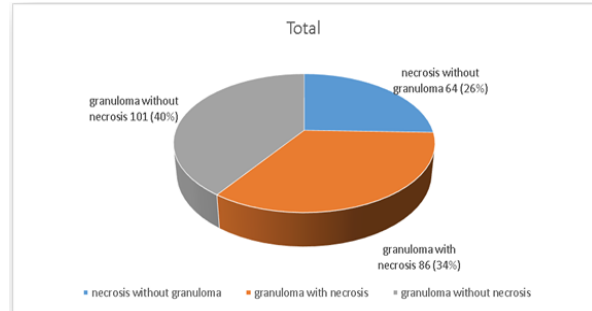


Fig-4: distribution of different cytomorphologies (n=251)

64(25.49%) cases had necrosis without granuloma. Out of those 64 cases 47(73.44%) showed AFB on ZN staining. Eighty six (34.26%) cases had epithelioid cell granuloma with necrosis & 101(40.24%) cases had epithelioid cell granuloma without necrosis. Results are elaborated in table-6

Table 6: Correlation of Cytomorphologies with AFB status in ZN stained smears (n=251)

Cytomorphology	AFB Present	AFB Absent	Total
Necrosis without granuloma	47(73.44%)	17(26.56%)	64
Granuloma with necrosis	48(55.81%)	38(44.19%)	86
Granuloma without necrosis	21(20.79%)	80(79.20%)	101
Total	116(46.22%)	135(53.78%)	251

r (Pearson correlation coefficient) = 0.43, p=<0.001, N=251 which indicates a medium association.

Total 19(6.23%) cases out of 305 were HIV positive. Among them 17(6.77%) showed cytomorphological features suggestive of tuberculosis but only 13 were positive for AFB in ZN stained smears.

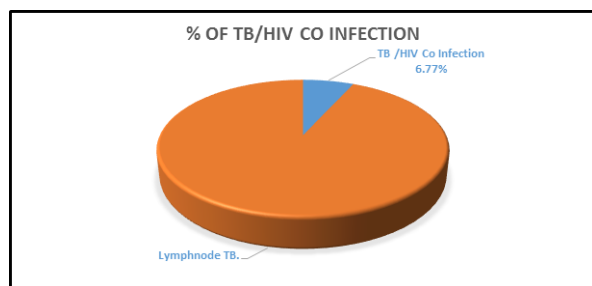


Figure 4: Distribution of TB/HIV co infection (n=251)

Table 7: Association of ICTC Status with AFB status (n=251)

ICTC Status	AFB Present	AFB Absent	Total
Reactive	13(76.47%)	4(23.53%)	17
Non-reactive	103(44.02%)	131(55.98%)	234
Total	116(46.22%)	135(53.78%)	251

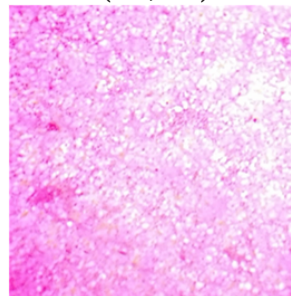
Pearson's Chi-Square = 6.715; df=1; P-value = 0.01. Association between rows and columns considered to be statistically significant

The final results are depicted in the table below

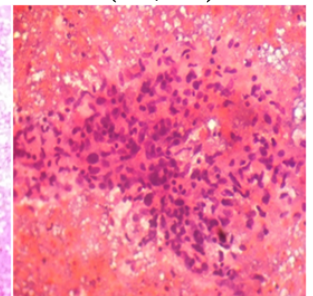
Table 8: Correlation of morphology on smears, AFB positivity and HIV positivity

Group	Morphology on Cytology	No. Of Cases n=251	AFB Positivity (ZN staining) (%)	HIV Positive
1	Necrosis only	71 (28.28%)	51 (71.8%)	9
2	Epithelioid cell granuloma with necrosis	88 (35.06%)	48 (54.54%)	4
3	Epithelioid cell granuloma with lymphoid cells	91 (36.25%)	18 (19.8%)	4
4	Only Polymorphs	1	1	
	Total	251	118 (47.01%)	17 (6.77%)

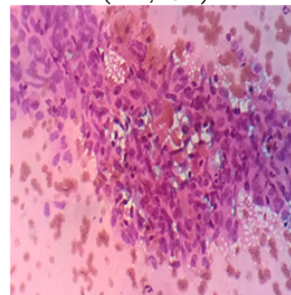
Photomicrograph showing necrosis without granuloma (PAP; 40 X)



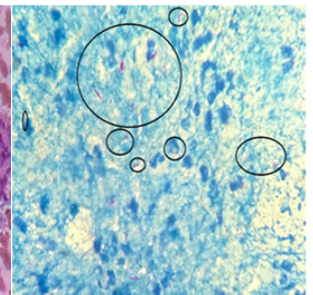
Photomicrograph showing granuloma with necrosis (PAP; 40 X)



Photomicrograph showing granuloma without necrosis (PAP; 40 X)



Photomicrograph showing Acid fast bacilli (ZN; 100 X)



DISCUSSION

Accurate and timely diagnosis together with effective TB treatment is the mainstay of TB care and control. Though culture and isolation of the organism is considered the gold standard in the diagnosis of most infections, culturing Mycobacterium tuberculosis as a diagnostic test is often not feasible in the clinical setup as the organism takes about 6 to 8 weeks to grow in conventional Lowenstein-Jensen medium.⁹ Though BACTEC system is a recent development for rapid detection of mycobacteria based on radiometric monitoring it is not cost effective. Therefore, quicker & accurate methods need to be established to diagnose TBL. In developing countries like India, the only practically available method for diagnosing extra pulmonary TB is direct smear microscopy for AFB of the sample from the lesion using ZN method but it has a low sensitivity.¹⁰ FNAC is an excellent diagnostic tool for diagnosis of tuberculosis in patients with lymphadenopathy. It is simple, safe, quick, reliable, accurate, minimally invasive, cost effective and most suitable in our country where tuberculosis is highly prevalent.¹¹ The cytological diagnosis of tuberculous lymphadenitis is usually based upon demonstration of conventional epithelioid cells, with or without multinucleated giant cells and with or without necrosis. Even in the absence of epithelioid cells, necrotic material has proved to be useful as it shows AFB positive

cases.⁹ In current study, out of 251 cases, 118 (47.01%) cases were positive for AFB. This was in concordance with the international data of a large-scale study of 328 cases, out of which 152 cases (46.4%) were positive for AFB.⁵

Similarly, our findings agree with Majeed et al.¹² who report 44% AFB positivity in ZN stained smear. Smear positivity for Mycobacteria by conventional ZN method was 39.13% (90/230) in a study by

Patel et al. and 26.7% (24/90) in study by Thakur et al.^{9, 13} Some studies report very high frequency of AFB positivity. Bezabih et al. reported 59.4% of overall AFB positivity, and Vignesh et al. reported 53.3% sensitivity for single AFB smear.^{14, 15} In present study AFB positivity was highest in caseous necrosis without epithelioid granuloma in 71.8% cases, which was 60% in a study by Patel et al. & 82.4% in the study by Almobarak AO et al.^{6, 16} AFB positivity was 54.54% in Epithelioid granuloma with caseous necrosis & lowest in epithelioid granuloma without necrosis 19.8% in present study.

Female gender was slightly more affected (64%) in current study and was in concordance with other studies.^{11, 12, 17} However, there was slight male predominance in a study of Bezabih et al. & Patel et al.^{14, 15} In current study 34.26% patients were of 20 years or below and 66.13% were below 30. This finding was in accordance with Bezabih et al.¹⁴ in which 69% were below 30 years & with other studies.^{11, 12} Based on the facts, it can be inferred that tuberculosis was more commonly seen in young population. Cervical lymph node was the most common site of involvement in studies followed by axillary lymph nodes.^{5, 11, 12, 14} Present study was also consistent with above studies in terms of cervical lymph node involvement (86.84%) as the most common anatomic site of granulomatous inflammation.

Moreover Human Immunodeficiency Virus (HIV) has long been known to cause severe Immunosuppression, leading to a rise in opportunistic infections, including tuberculosis. In HIV-negative individuals the lifetime risk of tuberculosis infection is 10%, while in HIV-positive individuals the risk is 7-12% per year.¹⁸ The association of this coinfection is synergetic, interactive and reciprocal, with significant impact on the course of the two pathologies. Coinfection is responsible for the increase in mortality rates, becoming a challenge for public health.¹⁹ According to a study by Neves et al. 391 patients with TB were notified, 84 (21.5%) of whom presented positive serology for HIV in the year 2009 in Brazil.⁶ An estimated 8.6% (range, 7.4-10%) of the incident TB cases in 2018 were among people living with HIV.²⁰ Another study showed that prevalence of TB/HIV co-infection was 31.25% in African countries, 17.21% in Asian countries, 20.11% in European countries, 25.06% in Latin America countries and 14.84% in the USA.²¹ In present study 19 cases out of 305 were HIV positive. Among them 17 (6.77%) showed cytomorphological features suggestive of tuberculosis but only 15 were positive for AFB on ZN smear. From these it can be concluded that though India have the largest number of tuberculosis cases: 27% of the global total,²⁰ TB/HIV co infection in India is much less compared to Western & African countries.

CONCLUSION

Fine needle aspiration cytology (FNAC) supplemented with special stains like ZN is very important investigation in the diagnosis of tuberculous lymphadenitis. The cytomorphological features suggestive of TBL are necrosis, epithelioid cell granuloma with necrosis & epithelioid cell granuloma with lymphoid cells. Among these necrosis show highest AFB positivity in ZN stained smears. A single smear showing only polymorphs was positive for AFB which concludes that even smears suggestive of suppurative inflammation should be searched for AFB in the respective ZN

stained smear. Also we can conclude from the above study that though India bears high burden of tuberculosis, HIV coinfection is less predominant in India as compared with western and African countries.

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