

“FNAC And ZN Staining: Simple And Effective Diagnostic Tool For Lymph Node Tuberculosis”

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Abstract

Introduction: Tuberculosis is one of the leading contagious and infectious disease, causing mortality and morbidity in India caused by *Mycobacterium tuberculosis*. TB involves many different sites. Tubercular lymphadenitis (TBLN) is one of the most common causes of lymph node enlargement in developing countries. FNAC has a very important role to diagnose TBLN.

Aim and Objective: Study was done to correlate various cyto-morphological findings in lymph node aspirate with AFB positivity in ZN staining.

Methods: The hospital-based observational analysis comprised 145 cytologically verified TB suspected cases. FNAC was performed and ZN staining was done. All the clinico-cytological findings were recorded and correlated with Acid Fast Bacilli (AFB) positivity. Four cyto-morphological patterns were categorized as- Pattern A- Epithelioid cell granulomas without necrosis. Pattern B- Caseous necrosis with epithelioid cell granulomas and giant cells. Pattern C- Caseous necrosis only. Pattern D- Caseous necrosis with suppuration.

Results: Among 145 cases, female predominance was noted with 96 cases. Number of cases in Pattern A, B, C and D were 44 (30.34%), 63 (43.45%), 12 (8.28%) and 26 (17.93%) respectively. AFB positivity was found in 42% cases.

Conclusion: FNAC is a simple minimally invasive, cost effective and safe procedure to diagnose tuberculosis lymphadenitis. Study of both cyto-morphological patterns and ZN staining for AFB can improve the diagnostic yield.

Keywords: AFB, Cytomorphology, Necrosis, ZN staining.

Date of Submission: 02-12-2025

Date of Acceptance: 12-12-2025

I. Introduction

TB is chronic granulomatous disease and is major cause of illness and mortality, particularly among underdeveloped nations.[1] The history of TB is as old as human race itself, making it one of the oldest diseases in humans.[2] Robert Koch discovered etiological agent of human tuberculosis, known as M.TB, more than 100 years ago, but this pathogen still poses a threat to human health.[3]

TB infections affect about 25% of the world's population. In 2023, TB was expected to have killed 1.25 million people, including 1.09 million HIV-negative individuals and 161,000 HIV-positive individuals.[4]

Pulmonary tuberculosis is more prevalent in men and older age groups, with a peak onset age of 20–40 years while Tubercular Lymphadenitis is more prevalent in women and younger age groups. [5,6]

According to reports, tuberculosis (TB) can infect nearly every organ except the hair, nails, and teeth.[7] Most common type of EPTB, accounting 35–40% of cases, is tubercular lymphadenitis, also known as scrofula. [8,9]

As TB is the major cause of morbidity and mortality in developing countries, it is essential to diagnose TB in early stage to initiate the treatment timely. FNAC offers good sensitivity and is an easy and affordable outpatient diagnostic technique for TB, it should be performed as the first diagnostic test in suspected cases of tuberculosis. Ziehl-Neelsen staining for Acid Fast Bacilli (AFB), culture, imaging studies, and molecular tests are other diagnostic methods available for diagnosis of Tubercular Lymphadenitis.[10]

Present study was undertaken to find out association of cellular components and necrosis with AFB positivity in tubercular lymphadenitis on aspiration cytology.

II. Material And Methods

A hospital based prospective study was done for 18 months in Department of Pathology, Muzaffarnagar Medical College, Muzaffarnagar (U.P.) after obtaining approval from the Institutional Ethical Committee.

All palpable and enlarged lymph nodes suspected as TB were examined out of which 145 cases were diagnosed as TBLN on FNAC.

In this study, total 145 patients having tubercular lymphadenitis were included. Proper history and clinical data such as site of aspiration, number of lymph nodes involved, nature of aspirates (blood mixed material, purulent/ pus, caseous or cheesy) were obtained. After informed consent, FNAC was performed and cytological smears were assessed for cytomorphology on Giemsa and ZN staining to determine acid fast bacilli (AFB).

Based on cytomorphological features, 4 patterns of TBLN were seen on Giemsa staining[11]-

Pattern A- **“Granulomatous Lymphadenitis”**- diagnosis was made when smear showed ‘Epithelioid cell granulomas without caseous necrosis’. Pattern B- **“Necrotizing Granulomatous Lymphadenitis”** smear showed ‘Epithelioid cell granulomas with caseous necrosis’. Pattern C- **“Only Necrotizing Lymphadenitis”** smear contained ‘Caseous necrosis without granuloma’. Pattern D- **“Necrotizing Suppurative Lymphadenitis”** the smear contained ‘Neutrophils with abundant necrosis’.

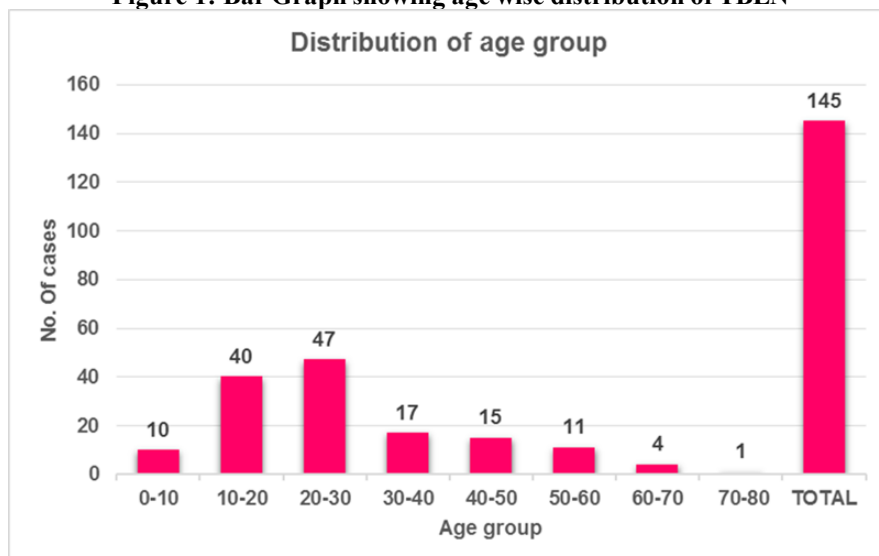
The findings of ZN staining was labelled as positive for acid fast bacilli (AFB), when there is presence of pink beaded and stumpy rod shaped organisms under oil immersion objective. Grading for AFB was done according to Kumar et al. under microscope on oil immersion (100X)- Grade 1- Smears with occasional bacilli. Grade 2- Smears with singly scattered AFB. Grade 3- Smears with large number of bacilli arranged in bundles. [12]

Statistical analysis- All the findings were recorded and calculated by averages and proportions. Excel data was prepared and analysed using SPS version 18.0. Chi-square test was done and p-value of <0.05 was considered statistically.

III. Results-

In 145 patients of tubercular lymphadenitis, examined patient’s age ranged from 6 months to 77 years with mean age of 28.8 ±15.3 years. The most common affected age group was third decade (47 cases, 32.41%). Least cases were noted in old age i.e. >60 years with only 5 cases (3.45%). (Figure-1)

Figure 1: Bar Graph showing age wise distribution of TBLN



Female preponderance was seen with male: female ratio of 1:2.2.

Although a combination of symptoms and signs were evident, fever and cough were noted to be the most frequent presenting complaints in most cases. Fever was noted in 105 (72.41%) cases followed by cough in 44 (30.34%) cases and weight loss in 38 (26.21%) cases. Night sweats was present in only 1 (0.69%) case.

Majority of the patients had symptoms for less than 3 months duration (59 cases, 40.69%). Symptoms for 3-6 months were seen in 21 cases (14.48 %) and more than 6 months in 18 cases (12.41 %).

Maximum aspirates were taken from cervical LN (108 cases, 74.48 %) indicating that the cervical LN are commonly affected by TBLN. This was followed by supraclavicular region (24 cases, 16.55 %). Axillary and inguinal region had least number of aspirates (9 cases, 6.21% and 4 cases, 2.76% respectively).

Single lymph node enlargement was seen among 110 (75.86%) cases and multiple lymph nodes among 35 (24.14%) cases. Well-defined lymph nodes were present among 91 (62.76 %) cases and ill-defined among 37 (25.52%) cases. Matted lymph nodes were present in 17 (11.72 %) cases. Lymph node was firm among 93 (64.14%) cases, soft among 38 (26.21%) cases and soft to firm among 14 (9.65%) cases.

After examination, FNA was done. The aspirate was blood mixed among 93 (64.14%) cases, pus in 41 (28.27%) cases and grey whitish among 11 (7.59%) cases. (Table-1)

Table 1 – Distribution of study population according to nature of aspirate and AFB positivity

Aspirate	Total cases (n)	AFB Positivity	
		No. of cases (n)	Percentage (%)
Blood Mixed	93	31	33.33
Pus	41	21	51.22
Grey Whitish	11	9	81.82
Total	145	61	

AFB- Acid Fast Bacilli
No.- Number

In our study, 4 main cytological patterns were seen-Pattern A was seen in 44 cases (30.34 %), pattern B among 63 cases (43.45%) and pattern C among 12 cases (8.28 %) and pattern D in 26 cases (17.93 %). (Table-2)

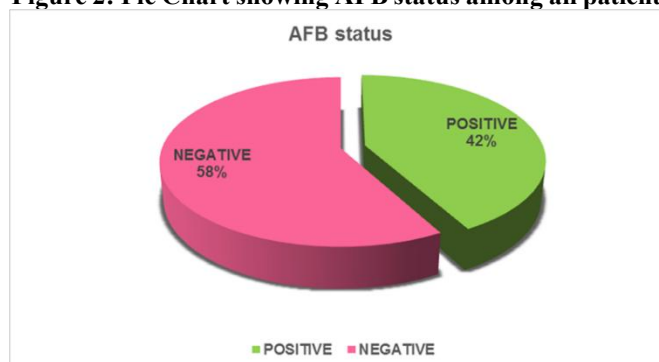
Table 2 – Distribution of study population according to cyto-morphological pattern and AFB positivity

	Patterns of TB Lymphadenitis	Total cases		AFB positivity	
		No. of cases (n)	Percentage (%)	No. of cases (n)	Percentage (%)
Pattern A	‘Granulomatous Lymphadenitis’	44	30.34	8	18.18
Pattern B	‘Necrotizing Granulomatous Lymphadenitis’	63	43.45	35	55.56
Pattern C	‘Only Necrotizing Lymphadenitis’	12	8.28	9	75.00
Pattern D	‘Necrotizing Suppurative Lymphadenitis’	26	17.93	9	34.62
	Total	145		61	

AFB- Acid Fast Bacilli
No.- Number

In these 145 cases of tubercular lymphadenitis, 61 (42.07 %) cases were AFB positive while in 84 (57.93 %) cases, AFB was not seen. (Figure-2)

Figure 2: Pie Chart showing AFB status among all patients



The AFB positivity was graded according to Kumar et al. on ZN staining. Grade 1 AFB positivity was seen among 41 (28.28 %) cases, grade 2 among 18 (12.41 %) cases and grade 3 was seen in 02 (1.38 %) cases.

AFB positivity among each aspirate is mentioned in Table-1. The chi-square statistic is applied and p-value was calculated as 0.00326 ($p < 0.05$), which was significant indicating that grey-white aspirate in LN is highly associated with presence of AFB.

Despite being least in number (12/145 cases), Pattern C (‘Only Necrotizing Lymphadenitis’) had the highest AFB positivity (9/12 cases, 75%). Pattern B (‘Necrotizing Granulomatous Lymphadenitis’) showed AFB in 35/63 cases (55.56%). Pattern- A (‘Granulomatous Lymphadenitis’) showed least (8/44 cases, 18.18%) AFB positivity and Pattern D (‘Necrotizing Suppurative Lymphadenitis’) showed 34.62 % AFB positivity (9/26 cases) (Table-2). Statistical correlation between various cyto-morphological patterns and AFB positivity was assessed using Chi-square test. (Chi-square value = 20.936, p -value = 0.000109) Result is significant as the p value ≤ 0.05 was considered significant for statistical significance. It denotes that there is association of AFB with caseous necrosis.

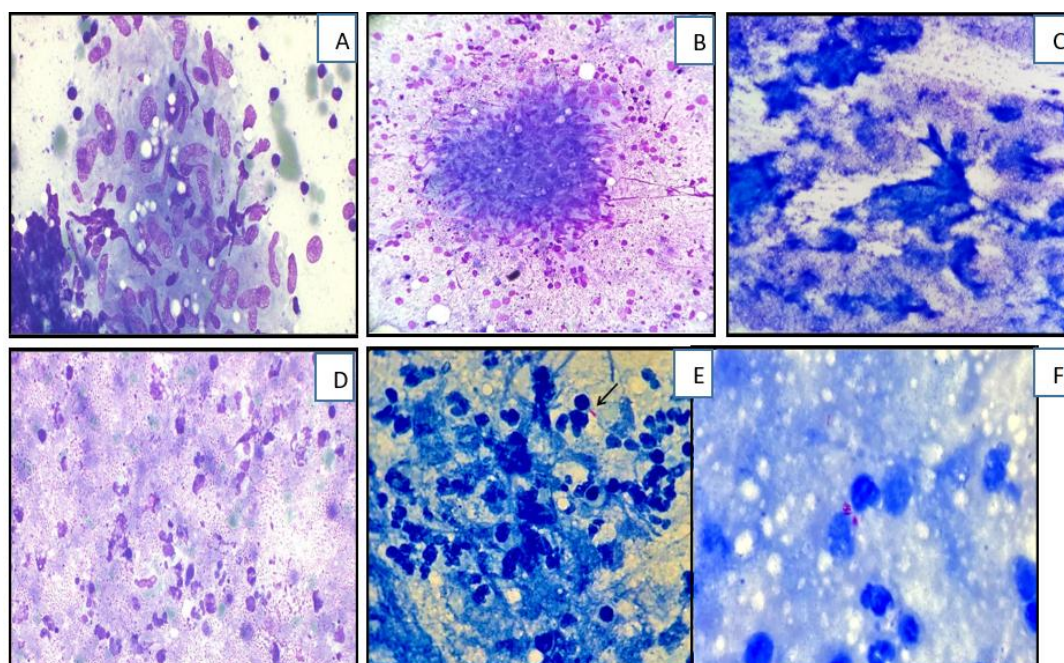


Image A- Photomicrograph showing granuloma comprising of epithelioid cells along with lymphocytes in Granulomatous Lymphadenitis (Giemsa stain,400X)

Image B-Granuloma along with necrosis in Necrotizing Granulomatous Lymphadenitis (Giemsa stain,400X)

Image C- Necrosis in Necrotizing Lymphadenitis (Giemsa stain, 100X)

Image D-Necrotizing Lymphadenitis with Suppuration showing neutrophilic infiltration (Giemsa stain, 400X)

Image E and F - ZN stain showing pink rod shaped acid fast tubercle bacilli (see arrow), (1000X), (Grade-1+, Image E) (Grade-2+, Image F)

IV. Discussion-

TBLN is still a most common extra-pulmonary manifestation of tuberculosis both in children and adults. In order to detect TB infection in the initial stages of the disease, there is necessity for early diagnosis and timely treatment. This can be done by epidemiological surveillance for timely formulation and implementation of an effective TB control program. Fine needle aspiration cytology has been used extensively in the diagnosis of tuberculous lymphadenitis and has become a first line diagnostic technique. Diagnosis of tuberculous can be established by demonstrating acid-fast bacilli (AFB) in FNA smears with Ziehl-Neelsen stain.[13]

We examined 145 cytologically diagnosed TB lymphadenitis cases during the study period, focusing on their cytomorphological features and AFB positivity.

In our study, female preponderance was seen with 100 females and 45 males making M: F ratio of 1:2.2. The most common age group was the third decade, comprising 32.41% of cases, with mean age of 28.8 $\pm$ 15.3 years. Similar findings were seen in study done by Dhaliwal D et al. with a mean age of 32.6 years. The majority of patients (26.4%) were in third decade, which was consistent with our analysis with female: male ratio of 1.01:1.[14] Study done by G S & Prasad C et al. also showed similar findings.[11] In majority of studies, women preponderance was seen. This could be because of various factors like females from low socioeconomic backgrounds have poor nutritional status, which makes them more susceptible for infection.[15]

The most common location of TBLN is cervical lymph nodes i.e. 74.48% cases, followed by supraclavicular (16.55%), axillary (6.21%) and inguinal lymph nodes (2.76%). Study done by Bohara S et al.[7] and G S & Prasad C et al. [11], also showed cervical LN most commonly affected.

In our research, aspirate was blood mixed among 64.14% cases, pus in 28.27% cases and thick whitish aspirate among 7.59% cases.

In the present study, Giemsa stained smear showed Pattern A (Necrotizing Granulomatous Lymphadenitis) among 43.45 % cases and Pattern B (Granulomatous Lymphadenitis) among 30.34 % cases. Pattern C (Only Necrotizing Lymphadenitis) was found among 8.28 % cases and Pattern D (Necrotizing Suppurative Lymphadenitis) among 17.93 % cases. Study done by G S & Prasad C et al., Mandawat et al. and Vashisht N et al. also showed similar results.[11,13,16]

In present study, out of 145 cases, 61 cases (42.07%) are AFB positive on ZN staining. Dhaliwal D et al. showed similar result (36.3%).[14] Similarly, Ahmed S et al. found overall 40.3% AFB positivity [15] and Bohara S et al. noticed 26.96% AFB positivity.[7]

Different studies show variation in AFB positivity which may be due to numerous factors which influence AFB positivity like selecting the best smear with a representative and suitable sample (more necrotic particles indicate greater positivity), using the right staining techniques, and the subjective differences of the examining pathologist, including the amount of time spent on each slide during examination, level of alertness, and experience. These could be the causes of the significant differences in AFB positivity observed in different studies.[7]

Pattern C (Only Necrotizing Lymphadenitis) has the highest AFB positivity of 75%, whilst Pattern B (Necrotizing Granulomatous Lymphadenitis) shows 55.56 % of AFB positivity. On the other hand, Pattern A (Granulomatous Lymphadenitis) shows least, only 18.18% AFB positivity and Pattern D (Necrotizing Suppurative Lymphadenitis) shows 34.62 % AFB positivity. Ahmed S et al. noticed smears showing ‘Only Necrosis without epithelioid cell granuloma’ showed maximum AFB positivity of 91.8% followed by ‘Epithelioid cell granuloma with necrosis’- 52.7%. ‘Epithelioid cell Granuloma without necrosis’ showed 7.9% positivity and ‘Poorly formed granuloma/small histiocytic clusters without necrosis’ showed the least positivity of 3.8%.[15]

Grey whitish aspirate showed 81.82% (9/11 cases) AFB positivity and blood mixed aspirate showed 33.33% AFB positivity (31/93 cases). Pus aspirate showed AFB positivity i.e. 51.22% (21/41 cases). Similar result was seen in other studies. G S & Prasad C et al. observed that AFB positivity was highest among cases which yielded cheesy & purulent material (69%), while blood mixed aspirate yielded lowest rate of AFB positivity (26%).[11]

Purulent aspirates are frequently diagnosed as suppurative lesions, but because of the high incidence of AFB positivity in these lesions, it is strongly advised to conduct ZN staining and a comprehensive search for epithelioid granulomas to increase the diagnosis of true- positive cases of TB. Misdiagnosis of tuberculosis as suppurative abscess may occur because of the lack of distinctive characteristics brought on by mixed inflammatory bacterial super-infections. The current study highlights the importance of understanding the clinical- pathological indicators of tuberculosis.[7]

Conflict of interest- Nil

Funding support- Nil

V. Conclusion-

Tuberculosis is a chronic granulomatous disease and is one of the most common causes of lymphadenopathy. Fine needle aspiration cytology (FNAC) is a common OPD procedure used as a diagnostic tool in suspected tubercular lesions. It is a cost effective and less time consuming process, creating very less distress to the patients. It is also a secure, minimally invasive, successful, feasible and convenient alternative to open biopsy of lymph node. The present study highlights that purulent to grey whitish aspirates and microscopic finding of suppuration and necrosis on FNAC in combination with clinical scenario of younger age group and combination of suggestive symptoms are highly suggestive of TB. ZN staining on cytology smears in the above scenario is very helpful for clinching an early diagnosis in resource-limited settings.

The diagnostic accuracy of FNAC can be further improved manifold when used in conjunction with other special techniques like bacteriologic culture, ultra-structural studies and molecular hybridization. The present study suggests that FNAC coupled with ZN staining for AFB is an excellent method for diagnosing tubercular lymphadenitis.

This study recommends microbiological evaluation in AFB negative cases. Since 10,000 – 100,000 mycobacterial organisms/mL of sample are required for AFB positivity in ZN staining, microbiological evaluation is required in AFB negative cases to confirm diagnosis of TB.[17]

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