

# Cytomorphological spectrum and Ziehl-Nelson staining in suspected tuberculous lymphadenitis: A study of 100 cases.

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**Keywords:** Tuberculosis, Cytology, AFB, Lymphadenitis

## Abstract

**Background:** Tuberculosis (TB) is the leading cause of death from a curable infectious disease. The situation is further worsened by a resurgence of the disease in immunocompromised AIDS cases. Lymphadenitis is the most frequent form of extrapulmonary tuberculosis. The diagnosis of tuberculous lymphadenitis remains challenging in spite of the availability of various diagnostic tools.

**Aims:** The present study aims at studying different cytomorphological patterns in suspected cases of tuberculous lymphadenitis and the efficacy of cytology and Ziehl-Nelson (ZN) staining in diagnosing tuberculous lymphadenitis in these different patterns.

**Methods:** One hundred cases clinically suspected of tuberculous lymphadenitis were studied (over a period of one year) with the aid of fine needle aspiration cytology (FNAC) complemented with AFB staining. The cytomorphological spectrum along with AFB positivity in different groups was compared.

**Results:** Maximum AFB positivity was seen in cases showing cytomorphological features consistent with a granulomatous lesion with necrosis (i.e. in 10 out of 21 cases; 48%). The difference in AFB positivity between various cytomorphological groups was found to be statistically significant ( $p < 0.01$ ) especially between cases showing caseating granuloma and those showing non-caseating granuloma or reactive lymphadenitis at 1% level of significance. The maximum sensitivity of ZN stain was found in cases showing caseating granuloma, and was nil in cases showing non-caseating granuloma and cases showing reactive lymphadenitis.

**Conclusion:** The maximum AFB positivity is seen in cases showing cytomorphological features consistent with a granulomatous lesion with necrosis. A combination of conventional techniques (FNA and ZN staining for AFB) and newer diagnostic techniques must be applied for the rapid and early diagnosis of tuberculosis in paucibacillary specimens to achieve maximum sensitivity.

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## Introduction

Tuberculosis (TB) is the leading cause of death from a curable infectious disease. Despite national and international tuberculosis control programs, TB, a treatable condition, is rampant in India. The situation is further worsened by a resurgence of the disease in immunocompromized AIDS cases.<sup>[1]</sup> Lymphadenitis is the most frequent form of extrapulmonary tuberculosis, usually occurring in the cervical region ("scrofula").<sup>[2]</sup>

The diagnosis of tuberculous lymphadenitis remains challenging in spite of the availability of various diagnostic tools. Conventional microbiological methods, like ZN staining and culture for *Mycobacterium tuberculosis*, are traditionally used in the diagnosis of tuberculous lymphadenitis.<sup>[3]</sup> However, none of these methods alone can diagnose all cases of TB lymphadenitis. Standard diagnostic algorithm for tuberculous lymphadenitis in India recommends FNAC with ZN staining for acid fast bacilli (AFB) in clinically suspected cases.<sup>[4]</sup> Detection of AFB by conventional microscopy is simple and rapid but lacks adequate sensitivity.

## Materials and Methods

The present prospective study was conducted in the Department of Pathology and Microbiology, Safdarjung hospital & VMMC (a tertiary care hospital), New Delhi. One hundred cases clinically suspected of tuberculous lymphadenitis were studied (over a period of one year) with the aid of FNAC complemented with AFB staining. Clinically suspected cases between 15 years and 65 years of age, referred to the Department of Histopathology were included in the study. An informed express consent of the patient was taken in each case. Relevant clinical history and findings were recorded. FNAC was performed in all the cases using a 22G needle. The material aspirated on FNA was used to make the smears. Air dried smears were stained immediately with Giemsa stain for cytomorphological study and ZN stain for AFB.

The cytological criteria for diagnosis of tuberculosis were taken as:

1. FNA cytology showing epithelioid cell granulomas with or without multinucleated giant cells and caseation necrosis.
2. Presence of AFB (by ZN stain) in smears showing necrosis only/non-caseating granulomas/acute suppurative lymphadenitis.
3. Cases showing necrosis only/non-caseating granulomas/acute suppurative lymphadenitis without demonstration of AFB were considered as suspicious of TB.

The efficacy of FNAC and ZN stain in different cytomorphological spectrums is compared.

## Result

In this prospective study one hundred cases clinically and cytologically suspected of TB LAP, between 15 years and 65 years of age were studied. FNAC yielded purulent material in 61% cases, hemorrhagic/blood mixed aspirate in 26% cases and a cheesy white aspirate in 13% cases. Microscopic examination of Giemsa stained FNA smears was done. Based on the cytomorphological features, the cases were divided into 5 groups (Table 1).

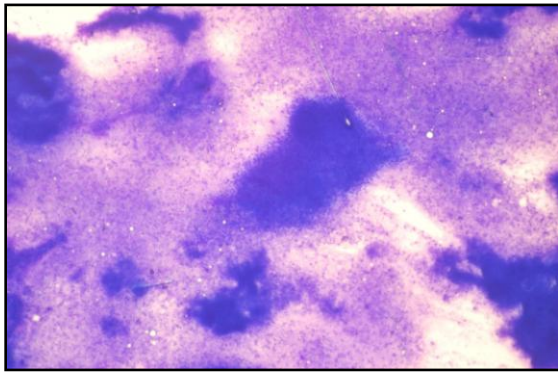
FNA smears showed that the most common cytomorphological pattern was acute suppurative lesion (Group I) in 40 out of 100 cases; caseating granulomatous lymphadenitis (Group II) was seen in 21 out of 100 cases; non-caseating granulomatous lymphadenitis (Group III) was seen in 3 out of 100 cases; necrotizing non-granulomatous lymphadenitis (Group IV) was seen in 20 out of 100 cases and reactive lymphadenitis (Group V) was seen in 16 out of 100 cases (Table 1) (Figure 1,2,3).

Correlation of the gross nature of aspirate on FNA was done with the cytomorphological pattern (seen microscopically). It was observed that in group I (ASL) the gross nature of aspirate was purulent in 54%, cheesy white in 23% and blood mixed in 15.3%

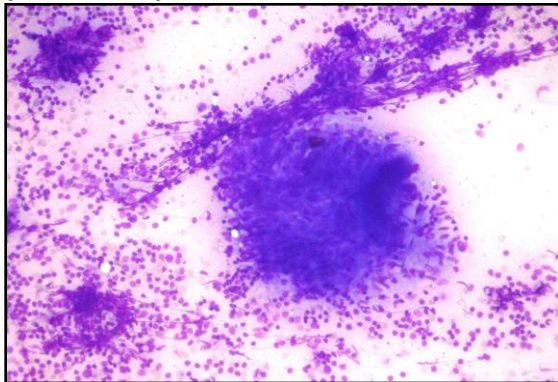
**TABLE 1: Correlation of the gross nature of aspirate on FNA with the cytomorphological pattern**

Groups	Cytomorphology	Nature of aspirate		
		Purulent	Cheesy white	Blood mixed
<b>Group I</b>	Acute suppurative lesion (ASL) (n =40)	33 (54%)	03 (23%)	04 (15.3%)
<b>Group II</b>	Granuloma with necrosis (n = 21)	10 (16.3%)	05 (38.4%)	06 (23%)
<b>Group III</b>	Granuloma without necrosis (n = 3)	01 (1.6%)	-	02 (7.6%)
<b>Group IV</b>	Necrosis only (n = 20)	14 (22.9%)	05 (38.4%)	01 (3.8%)
<b>Group V</b>	Reactive lymphadenitis (n = 16)	03 (4.9%)	-	13 (50%)
<b>Total</b>	100	61	13	26

cases. In group II (caseating granulomatous lymphadenitis), the gross nature of aspirate was purulent in 16.3%, cheesy white in 38.4% and blood mixed in 23% cases. In group III (non-caseating granulomatous lymphadenitis), the gross nature of aspirate was purulent in 1.6% and blood mixed in 7.6% cases; in group IV (necrotizing non-granulomatous lymphadenitis), the gross nature of aspirate was purulent in 22.9%, cheesy white in 38.4% and blood mixed in 3.8% cases; and in group V (reactive lymphadenitis), the gross nature of aspirate was purulent in 4.9% and blood mixed in 50% cases. Cheesy white aspirate was not seen in any of the cases belonging to group III and V. The more likely presence of ASL in purulent aspirates and reactive lymphadenitis in blood mixed aspirates was statistically significant ( $p < 0.01$ ) at 1% level of significance (Table 1).



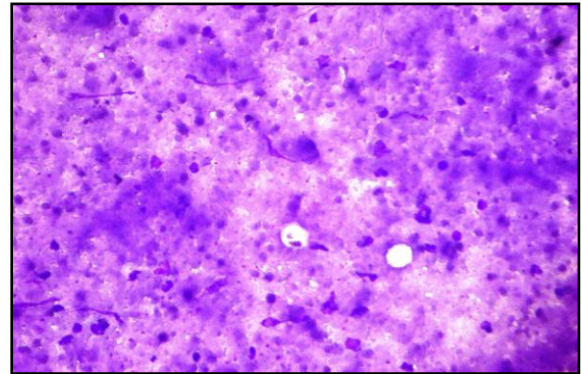
**Figure 1:** FNA smear showing caseating epithelioid cell granulomas against a necrotic background (Giemsa 200X)



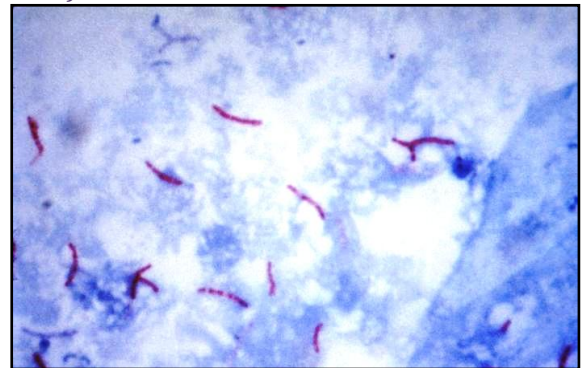
**Figure 2:** FNA smear showing caseating epithelioid cell granuloma against a reactive background (Giemsa, 400X)

Smears of each aspirate were stained for AFB using the ZN stain [Figure 4]. It was observed that in group I (ASL) the AFB positivity was seen in 20% cases; in group II (caseating granulomatous lymphadenitis),

the AFB positivity was seen in 48% cases; in group III (non-caseating granulomatous lymphadenitis), none of the cases showed AFB positivity; in group IV (necrotizing non-granulomatous lymphadenitis), the AFB positivity was seen in 35% cases and in group V (RLN) none of cases showed AFB positivity. Thus, maximum AFB positivity was seen in cases showing cytomorphological features consistent with a granulomatous lesion with necrosis (i.e. in 10 out of 21 cases; 48%). The difference in AFB positivity between various cytomorphological groups was found to be statistically significant ( $p < 0.01$ ) especially between cases showing caseating granuloma and those showing non-caseating granuloma or reactive lymphadenitis at 1% level of significance. However, the difference in AFB positivity between the groups showing ASL, only necrosis and caseating granulomatous lesion on smear was not statistically significant ( $p > 0.01$ ) at 1% level of significance. The maximum sensitivity of ZN stain was found in cases showing caseating granuloma and was nil in cases showing non-caseating granuloma and cases showing reactive lymphadenitis (Table 2).



**Figure 3:** FNA smear showing caseous necrosis, background shows scattered lymphocytes (Giemsa, 400X)



**Figure 4:** FNA smear showing acid-fast bacilli in a necrotic background (ZN stain, 1000X)

**TABLE 2: Cytomorphological spectrum and AFB positivity**

Group	Cytological Findings	AFB Smear	Cytodiagnosis rendered
I	Necrosis with neutrophils	08 (+)	TB abscess
		32 (-)	ASL
II	Epithelioid cell granulomas with caseating necrosis	10(+)	TBLN
		11 (-)	TBLN
III	Epithelioid cell granulomas without necrosis	0 (+)	-
		3 (-)	Suggestive of TB
IV	Necrosis only, +/- lymphocytes	7 (+)	TBLN
		13 (-)	Suggestive of TB
V	Reactive lymphoid cells with non-specific inflammation	0 (+)	-
		16 (-)	RLN
Total		25	

A comparison of cytological and mycobacteriological findings was done. 40 cases in the study revealed necrosis with neutrophils on microscopic examination of the smears. Out of these 40 cases, AFB positivity was seen in 8 cases only, in which a diagnosis of TB abscess was given, whereas in rest of the 32 cases a diagnosis of ASL was rendered. A diagnosis of TBLN was given in 21 cases showing epithelioid cell granuloma with caseating necrosis (group II); out of which 10 cases were also found to be AFB positive. Cytologically the smears revealed epithelioid cell granulomas without necrosis (group III) in 3 cases. The diagnosis on FNA in all these cases was given as suggestive of TB. All of the 3 cases showing epithelioid cell granuloma without necrosis were found to be negative for AFB on ZN stain.

Out of 20 cases showing only necrotic material (group IV), AFB positivity was seen in 7 cases. Hence a diagnosis of TBLN was given in these 7 cases. In rest of the 13 cases, a diagnosis suggestive of TB was rendered. 16 cases which were clinically highly suspicious of TB showed reactive lymphoid cells with non-specific lymphadenitis and were diagnosed as reactive lymphadenitis (group V) on microscopy. Since, there was a high degree of clinical suspicion of TB, these aspirates were also included in the study and processed further for ZN staining for AFB and PCR for *M. tuberculosis*. All 16 cases were found to be negative for TB (Table 2).

The age of patients of clinically suspected cases of TB lymphadenitis in the present study ranged from

15 years to 65 years with a mean age of 23.97 years. The peak incidence was seen in the age group 15–25 years. A female preponderance was observed, with a male: female ratio of 0.92:1. In this study, majority of the patients presented with a chief complaint of lymphadenopathy (65%) over a period of few weeks to months. Rest of the patients presented with complaints of lymphadenopathy with associated fever (16%) and lymphadenopathy with fever and cough (11%). History of contact with TB patients in the family/neighborhood was seen only in 07% .

The maximum number of patients presented with lymphadenopathy in the head and neck region with the highest incidence in the cervical group of lymph nodes (56%), followed in descending order of frequency by supraclavicular lymphadenopathy (18%), and submandibular and submental lymphadenopathy in 6% each. 10% of the patients presented with axillary lymphadenopathy and 04% with inguinal lymphadenopathy. All the cases showing reactive lymphadenitis (group V) on cytomorphological examination were found to be negative for *M. tuberculosis* on ZN stain.

## Discussion

TB continues to be a major public health problem in India, especially so, as there is now a lethal partnership of TB with HIV. Tuberculous lymphadenopathy is a very common presentation of the disease in all age groups. In our study, patients were in age group between 15 years and 65 years, with a peak incidence of 15–25 years. The mean age was 23.97 years.

Tuberculous lymphadenopathy can be seen in any age group, though according to TB India RNTCP status report 2010, the maximum numbers of cases were seen in age group of 15–65 years.<sup>[5]</sup> In studies by different authors most cases were seen in 2nd and 3rd decade of life, as is also the case in the present study.<sup>[6,7]</sup>

A female preponderance was observed in our study with male: female ratio of 0.92:1, which may be attributed to the under privileged condition of women in society in many parts of India.<sup>[8]</sup>

In our study, it was observed that the most commonly involved site of lymphadenopathy was cervical (56%) followed in descending order of frequency by supraclavicular (18%), axillary (10%), submandibular, submental (6%) and inguinal lymph nodes (4%). Most authors have documented that it is the upper jugular chain of cervical lymph nodes that are af-

ected frequently. The inguinal group of lymph nodes are least involved.<sup>[9-11]</sup>

The cytomorphological findings on FNA of the 100 cases included in the study ranged from acute suppurative lymphadenitis, caseating granulomatous lymphadenitis, non-caseating granulomatous lymphadenitis, necrotizing non-granulomatous lymphadenitis and reactive lymphadenitis.

A confident diagnosis of TB on morphology can be rendered when a combination of epithelioid cell granulomas and caseous necrosis with or without multinucleated giant cells is seen on FNAC, which is possible in only 60–70% cases.<sup>[12-14]</sup> The diagnosis is further confirmed by demonstration of AFB. Variable AFB positivity has been observed by different authors by ZN staining technique ranging from as low as 16% to 75.9%.<sup>[15,16]</sup>

Out of 100 cases included in the study, 25 were smear positive (i.e. AFB positive on ZN staining). AFB positivity using ZN stain was found to be maximum in smears showing granuloma with necrosis (group II) in 10 out of 21 cases (47.6%), followed in decreasing order by smears showing necrotic material only (group IV) in 7 out of 20 cases (35%) and ASL (group I) in 8 out of 40 cases (20%). None of the cases showing granuloma without necrosis (group III) and reactive lymphadenitis (group V) were AFB positive. The difference between various cytomorphological groups was found to be statistically significant ( $p < 0.01$ ) at 1% level of significance.

Tuberculous bacilli are usually found extracellularly in necrotic areas within or at the periphery of the granulomas. The proliferative granulomatous process is probably responsible for the low bacillary load.<sup>[17]</sup> Smears showing granuloma without necrosis have a low AFB positivity and cases showing reactive lymphadenitis have the least AFB positivity.<sup>[15]</sup> Most authors have reported highest AFB positivity in lesions showing granuloma with caseous necrosis, followed by necrosis only and ASL.<sup>[15,18]</sup> This is in concordance with the findings seen in our study. Thus cytomorphological appearance of FNA smears, especially in the absence of demonstrable AFB poses a major limitation in a definite diagnosis of TBLN.

Extrapulmonary tuberculosis encounters many problems like the paucibacillary nature of the samples, inadequate sample volume, apportioning of the samples for various diagnostic tests resulting in non-uniform distribution of microorganism, etc. All

these limitations reflect in the poor contribution of conventional techniques in the establishment of diagnosis of extrapulmonary tuberculosis. This has stimulated the application of polymerase chain reaction in the laboratory diagnosis of extrapulmonary tuberculosis.<sup>[19]</sup>

FNAC combined with clinical correlation is useful as a first line of investigation. It is also safe, simple, rapid and highly cost effective.

## Conclusion

Despite its well established usefulness in the diagnosis of tuberculous lymphadenitis, FNA and ZN staining has several limitations in its clinical applications especially when the presence of AFB is not proven.

A combination of conventional techniques and newer diagnostic techniques must be applied for the rapid and early diagnosis of TB in paucibacillary specimens to achieve maximum sensitivity.

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## Competing Interests

None declared

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