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## **Original Research Article**

# Cytomorphological patterns of tubercular lymphadenitis with special emphasis on acid fast bacilli (AFB) positivity: A tertiary hospital based study in Banda district of Uttar Pradesh

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#### **Abstract**

**Background:** Tubercular lymphadenopathy is the most common manifestation of extra pulmonary tuberculosis. Fine needle aspiration cytology (FNAC) is a safe, inexpensive method to diagnose tuberculosis which if coupled with Ziehl Neelson (Z.N) staining can give accurate results.

**Aim and Objective:** To categorize the cytomorphological patterns of tubercular lymphadenitis and estimate the Acid-fast bacilli (AFB)positivity in each pattern along with comparison of AFB positivity among all the four patterns.

Material and Methods: Total 292 patients, diagnosed of features, such as granulomatous lymphadenitis, chronic necrotising granulomatous lymphadenitis, chronic necrotising abscess and chronic necrotising lymphadenitis on cytology, suggestive of tubercular origin, were categorised as four distinct cytomorphological patterns respectively. All the cases were also simultaneously subjected for Z.N staining. Percentage of AFB positive cases in each pattern was calculated.

**Results:** Highest AFB positivity was observed in cases of chronic necrotising lymphadenitis (95.4%), followed by chronic necrotising granulomatous lymphadenitis (82.6%), chronic necrotising suppurative lymphadenitis (53.5%) and least in granulomatous lymphadenitis (1%). Overall AFB positivity was 53.4%.

Conclusion: Tubercular lymphadenopathy presents with distinctive patterns on cytology and is also variably positive for AFB staining depending on the immune status of the patient.

Keywords: Cytomorphological patterns, FNAC, Tubercular lymphadenitis, Acid-fast bacilli.

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# 1. Introduction

Extra-pulmonary tuberculosis constitutes 15-20% cases of T.B. Out of these, lymph node presents as the most common site in nearly in 35% of cases. Tubercular lymphadenopathy presents as solitary or matted lymph nodes or as cold abscess or multiple sinus formation and is readily amenable for FNAC which usually is the first line investigation for its diagnosis. Aspirated samples can be simultaneously used for AFB staining, for molecular tests such as CBNAAT (Cartridge based nuclear acid amplification test and True NAT, a variant of PCR (Polymerase chain reaction) and also for T.B culture, the latter two tests being used when AFB is

not visible in the cytology smears.<sup>2</sup> Due to the high incidence of tuberculosis in our country, presence of epithelioid cell granuloma on cytology along with caseous necrosis is regarded as the most significant diagnostic indicator of tuberculosis.<sup>3,4</sup> However cytological presentations of tubercular lymphadenitis can be highly variable, likewise in many cases, only epithelioid cell granuloma without accompanying necrosis is present, and many others can present with predominantly abundant caseous necrotic material with scant inflammatory infiltrate and no epithelioid cell granuloma formation. Few cases can also cytologically resemble as acute pyogenic abscess where abundant polymorphs, lymphocytes and histiocytes are present with

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only focal areas of necrosis in the background and no granuloma formation. In these cases, necrosis is the only clue of tubercular aetiology. In the latter two scenario, AFB positivity on Z.N staining is the standard diagnostic test of tuberculosis, which if negative, can further be confirmed by rapid molecular tests such as CBNAAT or True NAT.

T.B culture using L.J medium (Lowenstein Jenson medium) is traditionally considered as a gold standard diagnostic test for tuberculosis, but a major limitation of this test is the culture results taking 4-6 weeks' time for diagnosis.<sup>5</sup> Nucleic acid amplification tests (NAAT) employing PCR have evolved as a powerful tool for rapid, point on care detection of mycobacterium genome on cytology aspirates.<sup>6</sup>

In this study, we describe the different cytomorphological patterns of tubercular lymphadenitis, the variations in acid fast bacilli (AFB) staining in relation to the patterns and their detection by TrueNAT testing in a tertiary medical care facility of a comparatively less explored region of Banda district of Uttar Pradesh.

#### 2. Material and Methods

Our study is a retrospective cohort study in which we have examined 598 cases of lymphadenopathy on cytology over a study period beginning from September 2018 to June 2022.

### 2.1. Inclusion criteria

We have included total 292 cases of lymphadenopathy in our study which have features suggestive of tuberculosis on FNAC and have been finally diagnosed as tubercular lymphadenitis either on basis of positive Z.N staining or on further testing with TrueNAT on cytology aspirate or on follow up with ATT (Anti tubercular therapy). All these cases also had relevant clinical history, radiological findings and presentation suggestive of tubercular etiology.

### 2.2. Exclusion criteria

We have excluded cases of reactive lymphadenitis, acute suppurative lymphadenitis, metastatic lymphadenitis and cases of lymphoma diagnosed on cytology. Patients who were already taking ATT for previously diagnosed tubercular lymphadenitis were also excluded.

FNAC was done by 5 ml syringe of 23 gauge and the smears were stained by May Grunwald Giemsa (M.G.G) stain and Z.N staining was also performed on each case using 20% Sulfuric acid. (H<sub>2</sub>SO4) as the decolourising agent on one of the smears. For the Ziel nelson staining we cover the entire cytological smear with carbol fuschin for 5-7 minutes and heated intermittently from underside by using the spirit lamp till fumes start coming up then wash the slide with running tap water. Decolourisation of the smear was done using 20% H<sub>2</sub>So<sub>4</sub> (Sulfuric acid) till the smear becomes faint pink in colour. Again, staining of the smear with methylene blue

stain was done for 2 minutes then dried and mounted the smear.

All 292 cases diagnosed with tubercular lymphadenitis on cytology were further categorised in four distinct cytomorphological patterns:

- Pattern A (Granulomatous Lymphadenitis- Smears showing predominantly many or few epithelioid cell granulomas surrounded by a reactive population of lymphoid cells without presence of necrosis). (Figure 1a).
- 2. Pattern B (Chronic necrotising granulomatous Lymphadenitis- Smears showing epithelioid cell granulomas along with a background abundant caseous necrosis along with many intact or degenerated inflammatory cells such as neutrophils, lymphocytes and histiocytes). (**Figure 1**b).
- 3. Pattern C (Chronic necrotising abscess without granuloma formation Smears showing marked infiltration of intact polymorphs, lymphocytes, histocytes along with focal areas of caseous necrosis in the background. No granuloma is seen). (**Figure 1c**).
- Pattern D (Chronic necrotising lymphadenitis- Smears showing predominantly abundant caseous necrosis with scant inflammatory infiltrate comprising of degenerated neutrophils, lymphocytes and histiocytes. No granuloma is seen). (Figure 1d)

Percentage of cases falling in each pattern was calculated along with AFB positivity percentage in each pattern. Zero AFB /100 fields in oil immersion is considered negative. The cases in which AFB was negative were advised TrueNAT test. Serological status of the patients was not known.

#### 3. Results

Amongst all 598 cases of lymphadenopathy smears subjected to FNAC, 292 cases (48.8%) were detected as tubercular lymphadenitis. All 292 cases of tubercular lymphadenitis on cytology were further divided into four distinct (**Table 1**) Pattern A cytomorphological patterns. (Granulomatous lymphadenitis)-95 cases (32.3%), Pattern B (chronic necrotising granulomatous lymphadenitis)-75 (25.6%), Pattern C (chronic necrotising abscess without granuloma formation) –56 (19.2%) cases, Pattern D (Chronic necrotising lymphadenitis) – 66 (22.6%). AFB positive cases in each pattern was calculated. Total 156 patients were positive for AFB. Overall AFB positivity was 53.4%. (156/292 patients).

Highest AFB positivity was seen in cases of chronic necrotising lymphadenitis (Pattern D)- 95.4%, followed by chronic necrotising granulomatous lymphadenitis (Pattern B)- 82.6%, chronic necrotising abscess without granuloma (Pattern C) - 53.5% and least in granulomatous lymphadenitis (Pattern A)- 1%.

In the rest 136 cases, where AFB staining on smears was negative, were advised TrueNAT, a confirmatory rapid test for tuberculosis, in 128 patients, the TrueNAT detected T.B bacilli. In 8 patients, all diagnosed with granulomatous lymphadenitis (Pattern A) in whom the TrueNAT test came negative, those patients were followed up, after initiation of ATT, and showed resolution of symptoms, were finally diagnosed as tubercular lymphadenitis. (Table 2). The total 128 cases detected by TrueNAT included all the 26 AFB negative cases of chronic necrotising abscess (Pattern C), in 86 AFB negative cases of granulomatous lymphadenitis (Pattern A), in all 3 AFB negative cases of chronic necrotising lymphadenitis (Pattern D) and the rest 13 cases of chronic necrotising granulomatous lymphadenitis (Pattern B). (Table 3). Amongst all 292 patients of tubercular lymphadenitis, there was female predominance comprising of 186 female patients and 106 male patients. Most common age group affected was between 21-30 years age, comprising of 107 patients (36.6%) and with maximum number of females and males, followed by 11-20 years age group -88 patients (30.1%) and 31-40 years age group (13%). (Table 4). The median age group was 26.3 years. The commonest site was cervical region comprising of 261 cases (89.3%), followed by axillary region -24 cases (8.2%) and least in

inguinal region-5(1.7%) and 2 cases of generalised lymphadenopathy (0.68%). (**Table 5**). The aspirate obtained was pus like aspirate in 152 cases (52%), thick cheesy aspirate in 62 cases (21.2%), blood mixed aspirate - 78 cases (26.7%). (**Table 6**). In radiology, USG was available modality in all four patterns showing approximately similar findings like single to multiple enlarged lymphnodes, hypoechoic lesions with showing necrotic changes. No significant differences were found between different patterns.

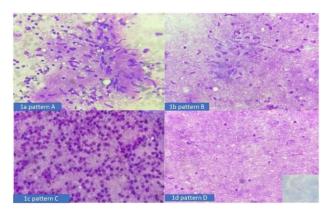


Figure 1: Different cytomorphological patterns in FNAC

Table 1: Number of cases in each cytomorphological pattern and number of AFB positive cases

Cytomorphological pattern	Total Number of cases (%)	Total number of AFB positive cases (%)	Number of AFB negative cases
Granulomatous Lymphadenitis (Pattern A)	95 (32.3%)	01 (1%)	94
Chronic necrotizing granulomatous Lymphadenitis (Pattern B)	75 (25.6%)	62 (82.6%)	13
Chronic necrotizing abscess without granuloma (Pattern C)	56 (19.2%)	30(53.6%)	26
Chronic necrotizing lymphadenitis (Pattern D)	66 (23%).	63 (95.4%)	03
	Total	156 AFB positive	136 AFB
	patients: 292	patients	negative patients

Table 2: Number of cases of tubercular lymphadenitis diagnosed by positive Z.N staining, TrueNAT and on follow up

Number of Cases of Tubercular Lymphadenitis Diagnosed in the Study by	Number of Cases
Respective Method	
Positive Z.N staining	156
TrueNAT	128
On follow up by ATT	08
Total patients	292

**Table 3**: Number of cases of tubercular lymphadenitis diagnosed by TrueNAT in each cytomorphological pattern

Cytomorphological Pattern	Number of Cases	Total Number of Cases in Each Pattern	Percentage of Cases Diagnosed by Truenat in Each Pattern
Pattern A- Granulomatous Lymphadenitis	86	95	90.5%
Pattern B- Chronic necrotizing granulomatous lymphadenitis	13	75	17.3%
Pattern C- Chronic necrotizing abscess with suppuration	26	56	46.4%
Pattern D- Chronic necrotizing lymphadenitis	03	66	0.05%
Total number of cases diagnosed by TrueNAT	128	Total no of cases-292	

**Table 4**: Age and gender wise distribution of patients

Age distribution of patients	Number of patients (%)	Number of females in each age group	Number of males in each age group
0-10	30 (10.3%)	21	09
11-20	88 (30.1%)	48	40
21-30	107 (36.6%)	69	38
31-40	38 (13.0%)	29	09
41-50	15 (5.1%)	10	05
51-60	09 (3.1%)	06	03
61-70	04 (1.4%)	02	02
71-80	01 (0.3%)	01	00
81-90	00 (0%)	00	00
91-100	0 (0%)	00	00
	Total number of patients: 292	186 females	106 males

**Table 5:** Site wise distribution of patients of tubercular lymphadenitis

Location	Number of patients (%)
Cervical	261(89.3%)
Axillary	24 (8.2%)
Inguinal	05 (1.7%)
Generalised Lymphadenopathy	02 (0.68%)
Total number of patients	292

**Table 6**: Nature of aspirate obtained in patients

Nature of aspirate	Number of patients (%)
Pus like aspirate	152 (52%)
Thick cheesy aspirate	62 (21.2%)
Blood mixed aspirate	78 (26.7%)
Total number of patients	292

# 4. Discussion

In our study, out of total 598 cases of lymphadenopathy, 292 cases (48.8%) were diagnosed as tubercular lymphadenitis, which indicates a high case burden of tuberculosis in the region. This might be attributed to a larger portion of rural population, poverty, malnutrition and lack of awareness, especially as Banda district was nominated as one of the country's 250 most backward districts in India by the Ministry of Panchayati Raj in 2006.7 Among all 292 cases of tubercular lymphadenitis, overall AFB positivity was 53.4%, with the highest AFB positive cases being observed in pattern D (chronic necrotising lymphadenitis), followed by pattern B- chronic necrotising lymphadenitis with granuloma formation, pattern C (chronic necrotising abscess without granuloma formation and markedly low AFB positive cases in Pattern A- granulomatous lymphadenitis, despite it having the highest number of cases amongst all the cases of tubercular lymphadenitis. The variable cytomorphological patterns of tubercular lymphadenitis are an important indicator of immune status of the patient which correlate with

the AFB positivity.8 Similar cytomorphological patterns of tubercular lymphadenitis was observed by Chand et al.9 and Dasgupta et al. 10 who reported cases of chronic necrotising lymphadenitis with suppuration as a distinct group with AFB positivity rate of 52.8% and 61% respectively, in this pattern, which is fairly similar to our study (AFB positivity -53.5%) observed in pattern C. The range of AFB positivity in tubercular lymphadenitis in many studies has been found varying from 44% to 71%<sup>1,8-12</sup> and in all of them, maximum AFB positive cases were seen in pattern with abundant necrosis without epithelioid cell granuloma and least in cases with epithelioid cell granuloma without necrosis, which is in concordance with our study. Low bacillary load is observed in granulomatous lymphadenitis (Pattern A) as there is little to no necrosis and many reactive lymphocytes are present which indicates a good immune response. Necrosis occurs when the activation of epithelioid macrophages is weak and hence the tubercle bacilli cause extensive tissue destruction.<sup>13</sup> In patterns of tubercular etiology where there is predominantly necrotic material, less inflammation and no granuloma, the bacillary load is high and the aspirate

obtained is thick cheesy white material, as was observed in our study especially in patterns B and D where abundant caseous necrotic material is present.

In pattern C (chronic necrotising abscess without granuloma formation), focal areas of necrosis in the background, without epithelioid cell granuloma along with numerous intact polymorphs was observed and hence it was classified as a distinct pattern. The focal necrotic material present in these smears can be easily be mistaken for the background proteinaceous exudate seen in acute pyogenic abscess in which the smooth amorphous appearance of caseous necrosis is difficult to ascertain, hence in these cases, clinical history and duration of the swelling is relevant. This pattern corresponded to the cold abscesses when the caseous necrotic material becomes liquified and the material aspirated is usually thin yellow pus. In our study, we came across 56 such cases and the AFB positivity in these cases was 53.5%. In these cases, the bacillary load was low and the search for AFB was very tedious. This could be attributed to the fact that the survival of tubercle bacilli in presence of superimposed acute suppurative inflammation and focal areas of necrosis, might be very low. This finding observed in our study stands contrary to the fact that the chance of finding AFB is high in cold abscesses, as stated by Mohapatra and Janmeja A.3 In pattern D (Chronic necrotising lymphadenitis without granuloma formation), smears showed abundant areas of caseous necrosis with few degenerated polymorphs, histiocytes, lymphocytes without definite granuloma formation. This pattern contributed to the highest number of AFB positive cases (95%), hence significantly correlating AFB positivity to presence of abundant necrosis. In all the AFB negative cases, True NAT test, a variant of TBPCR was advised for confirmation of tubercular aetiology.

In the current study, True NAT detected 128(94.1%) of cases as tubercular, while in rest of the 8 cases (all diagnosed with granulomatous lymphadenitis- Pattern A), it was indeterminate. This could be due to inadequate aspirated material, which might have got diluted with blood, leading to an indeterminate result. Based on cytological diagnosis and clinical presentation, all these eight patients were given ATT for 6 months, and were regularly followed up. All the patients showed gradual improvement, and were ultimately diagnosed as tubercular lymphadenitis. Numerous studies have established the utility of PCR on FNAC specimens as a highly sensitive and specific technique in the diagnosis of tubercular lymphadenitis. <sup>14,15</sup>

In our study, maximum patients were of 21-30 years age group (36.6%), followed by 11-20 years age group (30.1%) and 31-40 age (13%), 0-10 years (10.2%) and only one patient falling in 71-80 age group (0.3%). The age of patients ranged between 2- 72 years. Female patients were roughly twice the number of male patients comprising of 186 females and 106 males. Our findings are concordant with most of the studies.<sup>3,4,8-13</sup> In all these studies, the age incidence of

tubercular lymphadenopathy was maximum in 11-30 years age group, while the lowest incidence was found in the extremes of the age groups and there was female preponderance. Hemlatha et al.<sup>1</sup> has stated that in elderly it may be due to the development of cell mediated immunity against tubercle antigens in patients not suffering from any comorbid diseases such as diabetes mellitus, malnutrition while the extremely low incidence of tuberculosis in infants and children up to 2 years could be explained by the fact that while they may be infected by the tubercle bacilli, but the disease remains dormant, due to the still underdeveloped immunity, which might later manifest in the next few years.

Females tend to be more affected owing to the poor nutritional state along with low socioeconomic status especially those falling in the reproductive age group.

According to the site, cervical region was the most frequent site (89.3%), followed by the axillary region and the least cases in the inguinal region. According to Mohapatra and Janmeja, cervical lymph nodes are the most commonly affected site by extrapulmonary tuberculosis as they are the nearest site for the tubercle bacilli to lodge as they spread via hematogenous route by the reactivation of infection in the sites affected previously by primary tuberculosis such as lungs, tonsils, adenoids or sinonasal region.

In our study, solitary lymphadenopathy was the most common pattern observed in 141 cases (48.3%), while matted lymph nodes were present in 98 cases (33.5%), while the remaining 53 cases (18.2%) presented with matted lymph nodes with draining sinuses.

## 5. Conclusion

Our study shows a high case load of tubercular lymphadenitis, with an overall AFB positivity of 53.4% in Banda district of Bundelkhand region of Uttar Pradesh, which till date is one of the very few studies conducted in the region, along with describing the demographical profile of patients. Maximum AFB positivity was seen in cases with chronic necrotising lymphadenitis, while least was observed in granulomatous lymphadenitis. We have also described an additional cytological variant of chronic necrotising lymphadenitis with suppuration, which should not be mistaken for acute pyogenic abscess. Additionally, we have emphasized the diagnostic role of True NAT in AFB negative cases of tubercular lymphadenitis on cytology. Limitations of this study is the interobserver variation in identifying the true caseous material of tubercular etiology and confusing it with proteinaceous exudate of acute suppurative lesions as discussed earlier and also carefully searching the Acid-fast bacilli on Z.N. stained smears, which if not found can lead to a false negative result.

## 6. Source of Funding

None.

#### 7. Conflict of Interest

None.

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