

Cytomorphological Patterns of AFB Positive Tubercular Lymphadenitis: A Cross-sectional Study

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ABSTRACT

Introduction: Tuberculosis (TB) remains a global threat despite advancements in diagnostic technologies and treatment options. Tubercular lymphadenitis is the most common presentation of Extra Pulmonary Tuberculosis (EPTB) in developing countries, accounting for approximately 30-52% of cases. Fine Needle Aspiration Cytology (FNAC) plays a crucial role in the cost-effective and efficient outpatient diagnosis of tubercular lymphadenitis. Understanding the different cytomorphological patterns and Acid Fast Bacilli (AFB) density in tubercular lymphadenitis aids in early diagnosis, management, and reducing morbidity and mortality.

Aim: To analyse the various cytomorphological patterns observed in tubercular lymphadenitis, along with AFB density.

Materials and Methods: The present cross-sectional cytomorphological study was conducted between September and October 2021. Data from September 2020 to August 2021 were collected from the records of Shanthabai Devarao Shivaram (SDS) Tuberculosis Research Centre and Rajiv Gandhi Institute of Chest Diseases, a tertiary care hospital in Bengaluru, Karnataka, India. Out of a total of 128 patients with lymphadenopathy who underwent FNAC, four cases were excluded due to failed aspiration. The remaining 124 cases were available for analysis. Smears that exhibited features suggestive of tuberculosis were selected and categorised based on cytomorphological patterns into the following groups: Pattern-1 (Exudative), Pattern-2 (Epithelioid granuloma without necrosis), Pattern-3 (Epithelioid granuloma with necrosis), and Pattern-4 (Predominantly Necrosis without epithelioid granuloma).

Ziehl-Neelsen (ZN) stained AFB positive smears were further categorised into four groups based on mycobacterial load. The categories included scant (1-9 AFB in 100 fields), 1+ (10-99 AFB in 100 fields), 2+ (1-10 AFB per field), and 3+ (>10 AFB per field). Higher numbers indicated greater infectivity. Statistical analysis was performed using the Chi-square test. A probability level of less than 0.05 ($p < 0.05$) was considered statistically significant.

Results: Out of the 124 cases studied, 81 (65.32%) were females and 43 (34.68%) were males, with a female preponderance (1.9:1). The overall prevalence of tubercular lymphadenopathy was 89 cases (71.77%), with Pattern 4 (Acellular necrosis without granuloma) being the most common cytomorphological pattern 25 cases (28.09%) followed closely by Pattern-2 (Epithelioid cell granuloma without necrosis) 23 cases (25.84%). AFB positivity was observed in 57 cases (64.04%). Pattern-1 (Exudative pattern) showed 100% AFB positivity (21 out of 21 cases), while Pattern 4 (Predominantly necrosis without epithelioid granuloma) demonstrated 92% AFB positivity (23 out of 25 cases).

Conclusion: Understanding the various cytomorphological patterns in tubercular lymphadenitis is crucial for its diagnosis. Additionally, identifying AFB positivity, acid-fast bacilli distribution, and Bacterial Index (BI) in various patterns aids in planning early treatment and ensuring a favourable prognosis. FNAC serves as an easy, inexpensive, and quick procedure that can help avoid unnecessary surgical intervention or biopsy in the diagnosis of tubercular lymphadenitis.

Keywords: Acid fast bacilli, Epithelioid cell granuloma, Fine needle aspiration cytology

INTRODUCTION

Mycobacterium tuberculosis, a slender bacterium, is the causative agent of serious chronic pulmonary and systemic diseases worldwide. According to the World Health Organisation's Global TB report in 2021, approximately 10 million people become ill with tuberculosis each year, and 1.5 million die from the disease, with India contributing to one-fifth of the global incidence. Tubercular lymphadenitis accounts for a significant portion of extrapulmonary tuberculosis cases and can be diagnosed through various methods, including cytology, histopathology, Cartridge-based Nucleic Acid Amplification Test (CBNAAT) and culture. Mycobacterial culture remains the gold standard for definitive diagnosis [1-3]. Fine Needle Aspiration Cytology (FNAC) is a routine, rapid, inexpensive, and outpatient procedure that aids in the diagnosis of tubercular lymphadenitis, with a sensitivity and specificity of 88-96% [4].

The cytomorphological spectrum of tuberculous lymphadenitis reflects the disease stages and the immune status of the patient. Various cytological patterns, characterised by the presence of epithelioid cells and necrosis, can help establish a confident

diagnosis. Thus, understanding the different morphologies observed in tuberculous lymphadenitis smears contributes to early diagnosis and treatment [5]. If cytomorphological patterns could predict bacterial density, it would enhance diagnostic accuracy [6]. This knowledge facilitates the early initiation of appropriate treatment, reducing mortality and morbidity. The current study aimed to categorise tubercular lymphadenitis into different cytomorphological subsets using Fine Needle Aspiration (FNA), along with demonstration of AFB positivity in different cytomorphological patterns.

MATERIALS AND METHODS

This cross-sectional cytomorphological study was conducted in the Department of Pathology at SDS Tuberculosis Research Centre and Rajiv Gandhi Institute of Chest Diseases, Bengaluru, Karnataka, India a tertiary care hospital, over a period of one year from September 2020 to August 2021. Informed consent was obtained from the patients or their family members prior to the FNAC procedure, and complete medical history was obtained from available records.

Inclusion criteria: Cases with cytological features suggestive of tuberculous lymphadenitis, regardless of AFB status (positive or negative) were included in the study.

Exclusion criteria: Cases with insufficient material aspirated during FNAC, even with a strong clinical suspicion of tuberculosis were excluded from the study.

Study Procedure

Haematoxylin and Eosin (H&E), May Grunwald-Giemsa (MGG), and Ziehl-Neelsen stained smears that were available in the lab slide storage were used for analysis. Ziehl-Neelsen stained smears were specifically used for the identification of acid-fast bacilli.

Smears that exhibited features suggestive of tuberculosis were categorised into four cytomorphological patterns in the present study:

1. Exudative response
2. Epithelioid cell granulomas without necrosis
3. Epithelioid cell granulomas with necrosis
4. Predominantly acellular necrosis without granulomas [5]

ZN-stained AFB positive smears were further divided into four categories, similar to the original grading of AFB for sputum smears:

1. Negative (Zero AFB/100 fields)
2. Scanty or exact number of AFB seen (1-9 AFB in 100 fields)
3. 1+ (10-99 AFB in 100 fields)
4. 2+ (1-10 AFB per field)
5. 3+ (> 10 AFB per field) [7]

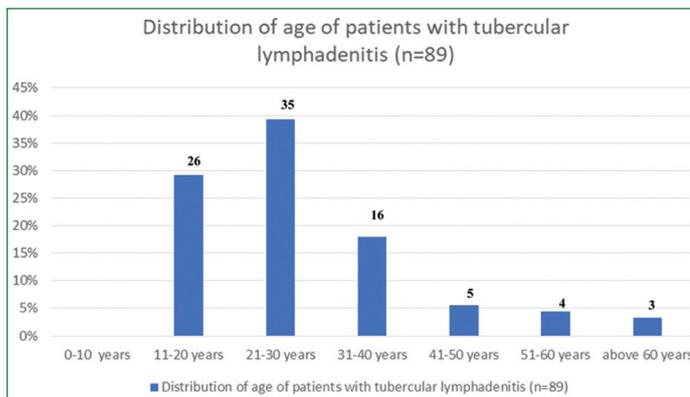
Selecting smears for ZN staining after screening MGG slides for foci of necrosis improves the AFB pick up rate from 42% to 92% [8].

STATISTICAL ANALYSIS

The data was entered into a computerised excel spreadsheet (Microsoft excel 2010) and analysed using Statistical Package for Social Sciences (SPSS) software version 21.0. Statistical analysis was performed using the Chi-square test. A probability level of less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS

A total of 128 cases with clinical suspicion of tuberculosis underwent FNAC in the present study. Four cases were excluded due to failure to aspirate material from lymph nodes, leaving 124 cases included in the study. The age of the patients ranged from five years to 65 years [Table/Fig-1]. Out of the 124 cases, 81 (65.32%) were females and 43 (34.68%) were males. Among the cases, 89 (71.77%) were diagnosed as FNAC suggestive of tuberculosis, 15 (12.10%) as metastatic deposits, 11 (8.87%) as reactive lymphadenitis, 6 (4.84%) as suppurative lymphadenitis, and 3 (2.42%) as lymphoma.



[Table/Fig-1]: Distribution of age of patients with tubercular lymphadenitis (n=89).

The frequency of tubercular lymphadenitis in the present study was 89 cases (71.77%), with the majority of cases seen in the 3rd decade of life 49 cases (39.5%). There was a female preponderance

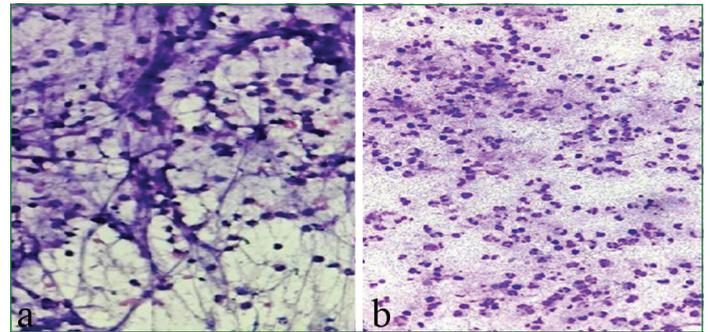
of 1.9:1. Swelling in the cervical region was the most common presentation, observed in approximately 100 cases (80%), and the most commonly aspirated node was the supraclavicular lymph node, seen in 54 cases (43.54%). AFB positivity was noted in 78 cases (62.90%), with 7 cases (5.64%) showing a reactive status for Human Immunodeficiency Virus (HIV).

Cytomorphologically, the cases of tubercular lymphadenitis were classified into four categories based on the presence of caseous necrosis, inflammatory cells (predominantly neutrophils), and epithelioid cells [Table/Fig-2].

Cytomorphological picture	No. of cases	Percentage (%)	AFB positive cases
Pattern-1 (Exudative response)	21	23.59	21 (100%)
Pattern-2 (Epithelioid cell granuloma with necrosis)	23	25.84	09 (39.13%)
Pattern-3 (Epithelioid cell granuloma without necrosis)	20	22.48	04 (20%)
Pattern 4 (Predominantly acellular necrosis without granuloma)	25	28.09	23 (92%)
Total	89	100	57 (64.04%)

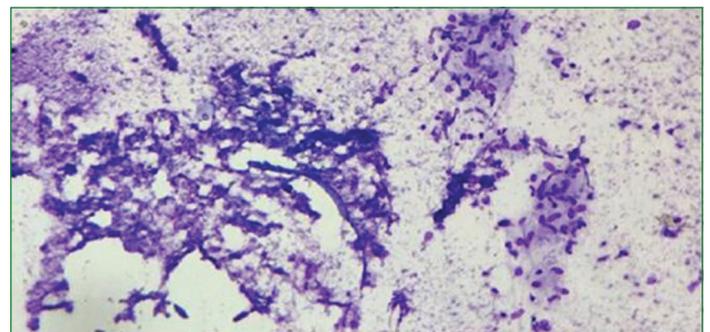
[Table/Fig-2]: Various cytomorphological patterns in tubercular lymphadenitis, number of AFB positive cases and its association.

Pattern 1-Exudative response: Observed in 21 cases (23.59%). This pattern predominantly consisted of an inflammatory infiltrate, mainly neutrophils, along with degenerated cells, a few lymphoid cells, and macrophages [Table/Fig-3a,b]. All cases in this pattern showed AFB positivity (100%), with a range of BI from scant to +3.



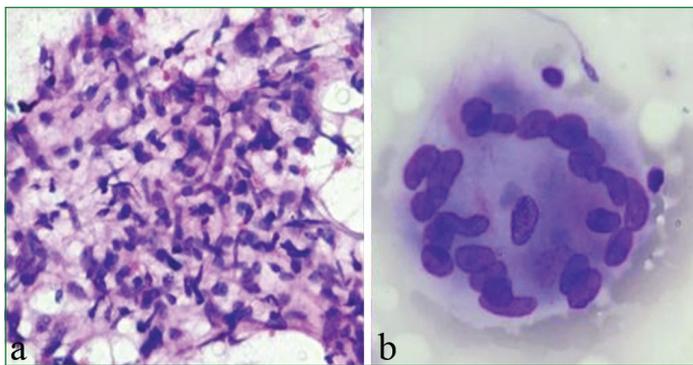
[Table/Fig-3]: a) Only inflammatory infiltrate, no granulomas. (H&E, 40X); b) Only neutrophils, no granulomas. (Giemsa, 40X).

Pattern 2-Epithelioid cell granulomas with necrosis: Noted in 23 cases (25.84%). This group exhibited epithelioid cells in well-formed or ill-formed granulomas, with evident necrosis and a polymorphous population of lymphoid cells, either present or absent [Table/Fig-4]. AFB positivity was observed in 9 cases (39.13%), with a range of BI from scant to +1.



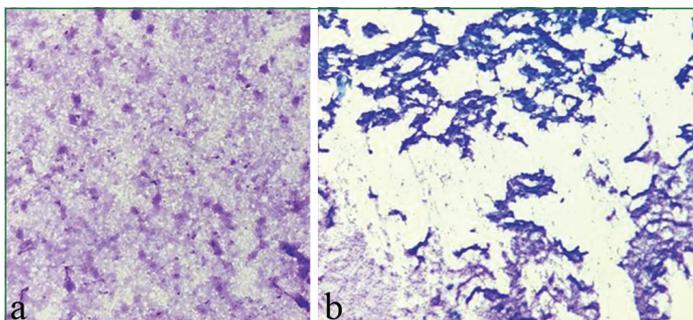
[Table/Fig-4]: Epithelioid cell granuloma with necrosis (Giemsa, 40X).

Pattern 3-Epithelioid cell granulomas without necrosis: Seen in 20 cases (22.47%). This pattern showed epithelioid cells in well-formed or ill-formed granulomas, or as single cells, in a lymphoid background without underlying necrosis [Table/Fig-5a,b]. AFB positivity was noted in all four cases (20%), with scant positivity.



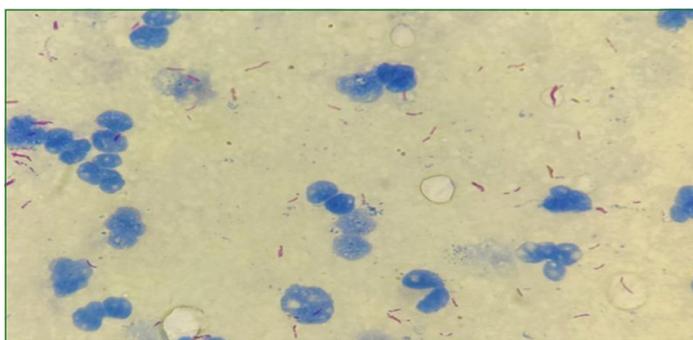
[Table/Fig-5]: a) Epithelioid cell granuloma without necrosis. (H&E, 100X); b) Epithelioid cell granuloma (Giemsa 100X).

Pattern 4-Predominantly acellular necrosis without granuloma: Observed in 25 cases (28.09%), making it the largest group [Table/Fig-6a,b]. AFB positivity was noted in 23 cases (92%), with a range of BI from scant to +3.



[Table/Fig-6]: a) Only necrosis, no granulomas. (H&E, 40X); b) Only necrosis, no granulomas. (Giemsa, 40X).

Among the 89 cases with cytological features suggestive of tubercular lymphadenitis, pattern 4 was the most common cytomorphological pattern, observed in 25 cases, followed by pattern 2 in 23 cases. AFB positivity was noted in 100% of cases displaying cytomorphological pattern 1 [Table/Fig-7], followed by 92% in cytomorphological pattern 4. The majority of cases with AFB positivity showed a BI of scant (80.70%) [Table/Fig-8].



[Table/Fig-7]: Acid Fast Bacilli (AFB)-(BI 3+) (ZN stain 100X).

Cytomorphological patterns (n=89)	AFB+(%) (n=57)	BI-Scant	BI-1+	BI-2+	BI-3+	p-value
Pattern-1 (n=21)	21 (100%)	15	02	01	03	p<0.001 Highly significant
Pattern-2 (n=23)	9 (39.1%)	06	03	00	00	
Pattern-3 (n=20)	4 (20%)	04	00	00	00	
Pattern-4 (n=25)	23 (92%)	21	01	01	00	
		46 (80.70%)	06 (8.77%)	02 (3.51%)	03 (5.26%)	

[Table/Fig-8]: AFB positivity in different tissue reaction pattern and bacteriological index according to AFB visualised under oil immersion.

S. No.	Study	Place of study/study duration	Total no. of cases	Most common lymph node involved	Various cytomorphology in tuberculous lymphadenitis n (%)			
					Necrotising suppurative lymphadenitis	Granulomatous lymphadenitis	Necrotising Granulomatous lymphadenitis	Necrotising lymphadenitis
1	Hemalatha A et al., [4]	Devraj Urs Medical College, Kolar	150	Cervical (122) (81.3%)	35 (22.66%)	30 (19.33%)	85 (56%)	-

DISCUSSION

In spite of progress in prophylaxis and treatment, India still has a high burden of tuberculosis, with extrapulmonary involvement in 15-20% of cases [9]. Lymph nodes are the most commonly affected site in extrapulmonary tuberculosis [1]. FNAC is an inexpensive and reliable tool for diagnosing tubercular lymphadenitis and can provide material for additional testing such as CBNAAT and culture and sensitivity. In endemic countries, granulomatous lymphadenitis is highly suggestive of tuberculosis but conditions like sarcoidosis, brucellosis, leprosy, malignancies like Hodgkin's lymphoma and metastatic neoplasms may also demonstrate epithelioid cell clusters [10,11]. In such cases, biopsy and CBNAAT can provide a definitive diagnosis [12]. In the present study, authors' have tried to stress the importance of various cytomorphological patterns noted in tubercular lymphadenitis, also importance of AFB positivity and BI in various cytomorphological patterns with the help of Zeihl-Neelsen staining.

In the present study, the most common age group affected by tubercular lymphadenitis was 21-30 years, which was concordant with studies done by Hemalatha A et al., (21-30 years, 30%), Gupta H et al., (21-30 years, 40%) and Chaudhari S et al., (21-30 years, 29%) [4,13,14].

The cytological patterns observed in tubercular lymphadenitis were classified into four categories in the present study. Pattern 4, characterised by acellular necrosis without granuloma, was the most common pattern [15]. Pattern 2, which showed epithelioid cell granulomas with necrosis, was the second most common pattern. Pattern 1, an exudative response with inflammatory infiltrate, and Pattern 3, epithelioid cell granulomas without necrosis, were less common. Studies which described four cytological patterns include Hemalatha A et al., Chaudhari S et al., and Paliwal N et al., [4,14,16]. The most common site involved was the cervical region, also in line with previous studies [Table/Fig-9] [2,4,6,14,16,17].

AFB positivity was observed in 64.04% of cases, with the highest positivity seen in Pattern 1 (100%) followed by Pattern 4 (92%). This suggests that AFB positivity is more likely in cases with an exudative response or acellular necrosis without granuloma, which could be attributed to early stages of disease, immunological status of patient, anti tubercular treatment and AFB concentration (To be detected by light microscopy, AFB has to be 103-106/mL of the material) [17]. Pradhan A et al., and Laishram RS et al., show maximum AFB positivity in cases showing caseating necrotising lymphadenitis similar to our findings [6,18].

Overall, the findings of the present study are consistent with previous studies and highlight the importance of different cytological patterns and AFB positivity in the diagnosis of tubercular lymphadenitis.

2	Paliwal N et al., [16]	LRS Institute of TB and Respiratory Diseases, New Delhi/Jul-Oct 2010	318	Cervical (286) (90%)	96 (30.1%)	45 (14.3%)	52 (16.4%)	125 (39.2%)
3	Chaudhari S et al., [14]	VCSGGMC and RI, Uttarakhand/Jan 2010-Jan 2015	465	Cervical (465) (100%)	23 (5%)	141 (30.3%)	240 (51.6%)	56 (12%)
4	Vashisht N et al., [17]	Lokmanya Tilak Municipal Medical College and General Hospital, Sion, Mumbai/Jan-Jun 2015	500	Cervical (436) (87.2%)	25 (5%)	112 (22.4%)	318 (63.6%)	45 (9%)
5	Pradhan A et al., [6]	BP Koirala Institute of Health Sciences, Dharan/Jan-Dec 2010	203	Cervical (126) (61.06%)	43 (21.18%)	37 (18.22%)	92 (45.32%)	31 (15.27%)
6	Mitra SK et al., [2]	BRD Medical College, Gorakhpur, Uttar Pradesh/June 2014-July 2015	180	Cervical (144) (80%)	-	53 (29.5%)	72 (40%)	55 (30.5%)
7	Present study	SDS Tuberculosis Research Centre and Rajiv Gandhi Institute of Chest Disease/Sep 2020-Aug 2021	124	Cervical (99) (79.8%)	21 (23.59%)	20 (25.84%)	23 (22.47%)	25 (28.09%)

[Table/Fig-9]: Comparison of cytomorphological patterns in various studies [2,4,6,14,16,17].

Limitation(s)

The present study has a limitation in that FNAC may not establish conclusive evidence in rare cases where only necrosis is present and AFB is negative. Such challenging cases might be missed and require confirmation through excision biopsy or culture if clinically indicated.

CONCLUSION(S)

Understanding the various cytomorphological patterns in tubercular lymphadenitis is of utmost importance for diagnosing TB lymphadenopathy. Additionally, identifying AFB positivity, the distribution of acid-fast bacilli, and bacterial index in various patterns provides valuable information for planning early treatment, improving prognosis, staging the disease, and assessing the patient's immune status. FNAC is an easy, inexpensive, and quick procedure that can be utilised to avoid unnecessary surgical interventions/biopsies in the diagnosis of tubercular lymphadenitis.

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