

A Clinicopathological Study of Fine Needle Aspirates of Lymph Nodes from Patients with Suspected Tubercular Lymphadenopathy: Analysis of 640 Cases from a Tertiary Health Care Centre in North India

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ABSTRACT

Introduction: Fine needle aspiration cytology (FNAC) of lymph nodes is an out-patient, simple and, quick, cost-effective procedure that helps in the diagnosis of tubercular lymphadenopathy. This technique has high sensitivity and specificity which in addition to the establishment of cytological diagnosis, also helps to perform ancillary tests like ZN staining for AFB Bacilli and GeneXpert MTB/RIF Assay.

Aims: To study the clinical and cytopathological features of fine-needle aspirates of lymph nodes from patients with suspected tubercular lymphadenopathy along with the role of ZN staining And GeneXpert assay in diagnosis.

Materials and Methods: In this study 1123 patients presenting with lymphadenitis presenting to the cytopathology lab for FNAC of lymph nodes between May 2018 to May 2021 were included. Out of 1123 patients presenting with lymphadenopathies, there were 640 cases of tuberculous lymphadenitis. The clinical and cytopathological findings of these 640 cases were analyzed.

Results: Most cases of tubercular lymphadenopathy were of the adult age group (20-59 years) and showed a female predominance. The most commonly involved lymph node was the cervical group. The most common clinical feature observed was cough (68.5%). Among the four cytomorphological patterns observed, maximum cases showed epithelioid cell granuloma with necrosis. Ziehl- Neelsen staining showed overall AFB positivity of 40.3%. Smear showing necrosis without epithelioid cell granuloma showed maximum AFB positivity among the four patterns. GeneXpert MTB/RIF Assay showed an overall detection rate of 84%.

Conclusion: FNAC is a simple, cost-effective, outpatient technique with high diagnostic accuracy in cases of tubercular lymphadenopathy which can be coupled by Ziehl Staining for Acid-fast bacilli and GeneXpert MTB/RIF Assay. However, in smear-negative cases, it poses a diagnostic dilemma, thus in a developing country like India with a high prevalence rate of tuberculosis, FNAC coupled with Z.N. staining and new molecular tests like GeneXpert MTB/RIF assay should be included in the first-line investigation in cases with tubercular lymphadenopathy.

Keywords: Tuberculosis, Fine Needle Aspirate Cytology (FNAC), Necrosis, Epithelioid cells, Cytopathological pattern, Ziehl-Neelsen Staining, GeneXpert Mycobacterium/Rifampicin (MTB/RIF) Assay

INTRODUCTION

Tuberculosis (TB) is a chronic granulomatous infection endemic disease in India caused by mycobacterium tuberculosis. Extrapulmonary TB which has less occurrence as compared to pulmonary TB consists of around 20% of cases in India. The most common site affected in extrapulmonary TB is the lymph nodes (1). Worldwide an average of 10 million people was affected by TB in 2019 while 2.69 million were affected by TB in India alone (2).

The diagnosis of tubercular lymphadenopathy can be made by fine-needle aspiration cytology combined with staining for acid-fast bacilli and newer methods like PCR- based amplification of mycobacterium DNA (3).

Fine-needle aspiration (FNA) cytology is minimally invasive and cost-effective. It is an outpatient diagnostic procedure, nevertheless, the sensitivity and specificity are variable, in the diagnosis of tubercular lymphadenopathy (4). The Ziehl-Neelsen (ZN) staining technique for acid-fast bacilli (AFB) is a simple, cheap, and rapid method. However, it has variable sensitivity, ranging from 20% to 43% (5).

GeneXpert MTB/RIF Assay is a new diagnostic method for the detection of pulmonary and extrapulmonary Tuberculosis which is based on the principle of real-time polymerase chain reaction (RT-PCR). Results are obtained in less than two hours and a has high sensitivity and specificity (6).

AIMS AND OBJECTIVES

- 1) To study the clinical features of patients suspected of tubercular lymphadenopathy
- 2) To study the cytopathological features of fine-needle aspirates of lymph nodes from patients with suspected tubercular lymphadenopathy.

- 3) To study the role of ZN Staining and GeneXpert MTB/RIF Assay as a first-line investigation in the diagnosis of patients with suspected tubercular lymphadenopathy.

MATERIAL AND METHODS

This study was a three-year prospective study which was conducted in the Department of Cytopathology, and Department of Microbiology, Jawaharlal Nehru Medical College and Hospital, Aligarh Muslim University, Aligarh, India. A total of 1123 patients presenting with lymphadenopathy underwent FNAC out of which 640 cases were found to be of tubercular etiology. Relevant clinical history and investigations were obtained and informed consent was taken. Smear was made for cytopathological examination and Ziehl Nelson Staining for Acid Fast Bacilli was done on these cases. Residual sample aspirated were sent for GeneXpert MTB/RIF Assay analysis in the department of Microbiology.

OBSERVATIONS

Most cases of tubercular lymphadenopathy were of the adult age group, that is 20-59 years (359-56%). 153 cases (24%) were of the pediatric age group or less than 13 years of age while 77(12%) were adolescents (14-19 years). Only 51 patients were above 60 years of age.

Out of a total of 640 cases in the study group, females comprised 378 cases (59.1%) while 262 were males (40.9%). The ratio between female and male patients was 1.4:1.

The most common site of lymph node involvement was found to be cervical with 259(40.9%) as depicted in table I.

Four cytomorphological patterns were observed. The most common pattern was epithelioid cell granuloma with necrosis consisting of 197 cases (30.8%) (Table II).

TABLE I: DISTRIBUTION OF CASES ACCORDING TO THE SITE OF LYMPHADENOPATHY

Location	Number of Cases	Percentage (%)
Cervical	259	40.4
Submandibular	100	15.7
Submental	67	10.4
Supraclavicular	64	10.0
Axillary	52	8.2
Infraclavicular	60	9.4
Inguinal	38	5.9
Total	640	100

TABLE II: DISTRIBUTION OF CASES ACCORDING TO CYTOMORPHOLOGICAL PATTERN

S. No	Cytomorphological Pattern	Number of Cases	Percentage (%)
a.)	Epithelioid cell Granuloma With Necrosis	197	30.8
b.)	Epithelioid cell Granuloma Without Necrosis	164	25.6
c.)	Necrosis Without Epithelioid cell Granuloma with Neutrophilic Infiltrates	148	23.1
d.)	Poorly Formed Granuloma/ Small Lymphohistiocytic clusters	131	20.5
	Total	640	100

a) Pattern1: Epithelioid cell granulomas with necrosis

This was characterized by well-formed epithelioid cell granulomas consisting of epithelioid histiocytes, multinucleated giant cells usually of Langhans type, inflammatory cells, and necrosis in the background. The epithelioid cells had elongated vesicular nuclei, fine nuclear chromatin, and pale cytoplasm. This was the most common pattern on FNAC seen in 197 smears suspected of tubercular lymphadenopathy (30.7% of the total) (Figure 1).

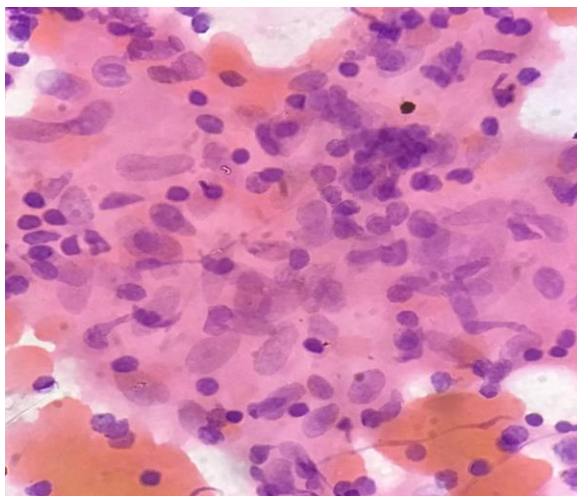


Figure 1: Well formed epithelioid cell granuloma in a background of necrosis (H and E X40)

b) Pattern 2: Epithelioid cell granulomas without necrosis

In this pattern, mainly well-defined epithelioid cells granulomas were seen, admixed with chronic inflammatory cells but without definite necrosis in the background, this pattern was seen in 164 cases (25.6%) (Figure 2)

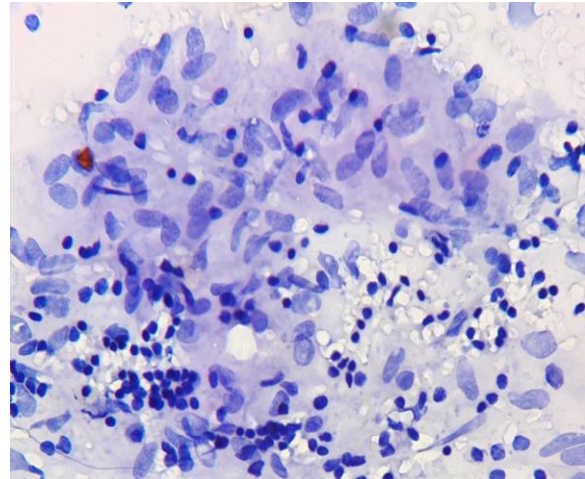


Figure 2: Well formed epithelioid cell granuloma without necrosis (Pap X40)

c) Pattern 3: Necrosis without epithelioid cell granulomas with neutrophilic infiltrates

Aspirates from 148(23.1%) patients were marked by the presence of necrosis, occasional epithelioid cells, macrophages and a prominent neutrophilic infiltrate (Figure 3).

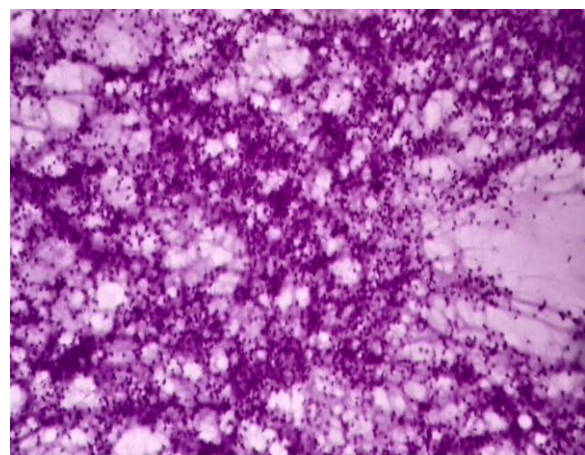


Figure 3: Necrosis without epithelioid cell granulomas with neutrophilic infiltrates (H and E X40)

d) Pattern 4: Poorly formed granulomas/ small lymphohistiocytic clusters

Ill-defined granulomatous aggregates were identified in 131(20.4%)

smears. Epithelioid cells and/or occasional giant cells could be detected in a few foci. In the background, a mixed population of lymphoid cells, plasma cells, and polymorphs are seen in variable proportions. Necrosis was usually absent in this pattern (Figure 4).

The most common presenting complaint was cough (439 cases, 68.5%) while least was loss of appetite (256 cases, 40%)(Figure 5).

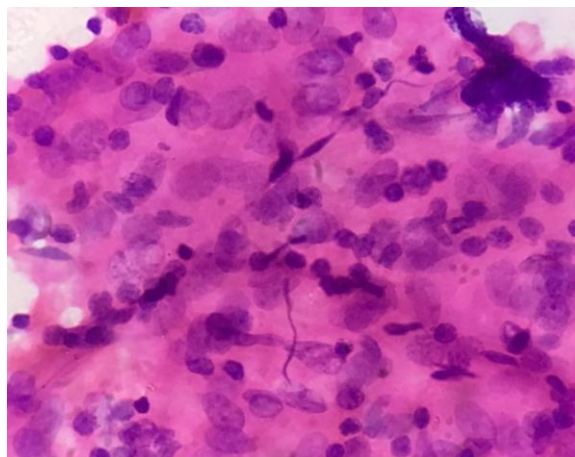


Figure 4: Poorly formed granulomas with few lymphohistiocytic clusters (H and E X40)

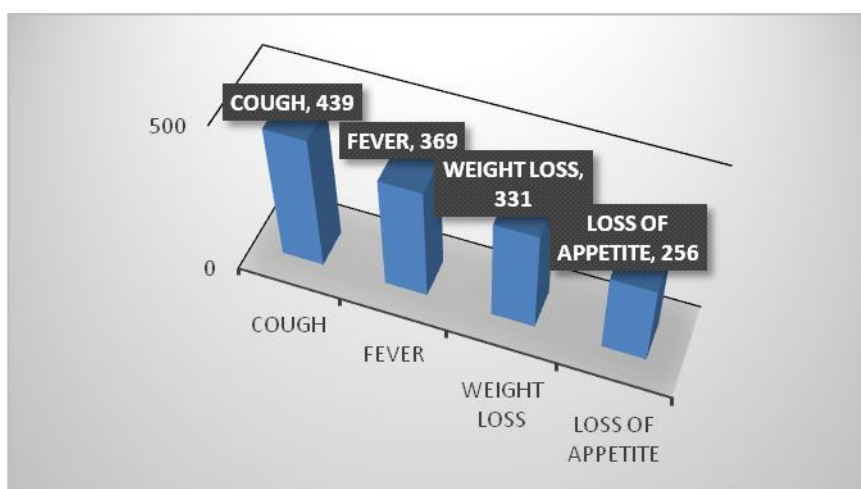


Figure 5: Distribution of cases according to predominant clinical history

Maximum AFB positivity (91.8%) was seen in smears from lesions having only necrosis without epithelioid cell granuloma followed by smears showing epithelioid cell granuloma with necrosis (52.7%). The least positivity (3.8%) was seen in smears

showing poorly formed granuloma/small histiocytic clusters without necrosis. The overall AFB positivity was found to be 40.3% (Figure 6) and the positivity rate by GeneXpert was found to be 84% as depicted in Table III.

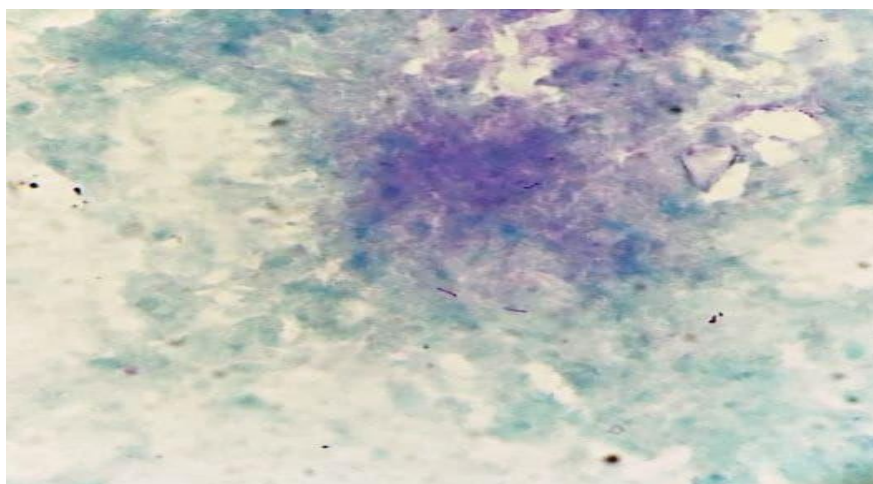


Figure 6: Several thin elongated red coloured bacilli (acid fast bacilli) against a bluish background. ZN Stain (X100, oil immersion)

TABLE III: DISTRIBUTION OF CASES ACCORDING TO CYTOMORPHOLOGICAL PATTERN AND RESULTS OF ZN STAINING AND GENEXPERT MTB/RIF ASSAY

S. No	Cytomorphological Pattern	Number of cases		GeneXpert assay		Total cases
		AFB Positive	AFB Negative	MTB/RIF		
				Positive	Negative	
a.)	Epithelioid cell Granuloma With Necrosis	104 (52.7%)	93 (47.3%)	189(95.9%)	8(4.1%)	197(30.8%)
b.)	Epithelioid cell Granuloma Without Necrosis	13 (7.9%)	151 (92.1%)	105(64%)	59(36%)	164(25.6%)
c.)	Necrosis Without Epithelioid cell Granuloma with Neutrophilic Infiltrates	136 (91.8%)	12 (8.2%)	141(95.2%)	7(4.7%)	148(23.1%)
d.)	Poorly Formed Granuloma/ Small Lymphohistiocytic clusters	5 (3.8%)	126 (96.2%)	103(78.6%)	28(21.4)	131(20.5%)
	Total	258 (40.3%)	382(59.7%)	538 (84%)	102(16%)	640(100%)

RESULTS

The present study was a 3-year prospective study conducted in the Department of Pathology, Jawaharlal Nehru Medical College and Hospital (JNMCH), Aligarh Muslim University (AMU), from 2018 to 2021. A total of 1123 patients presented with lymphadenopathy during this period and were advised to undergo Fine needle aspiration. In 640 patients, a tubercular etiology was clinically suspected. Smears made from these aspirates were stained with Haematoxylin and Eosin stain (H and E) and/or Papanicolaou (PAP) stain and analysis was done. 359 patients (56.0%) were of the adult age group (20-59 years) followed by 153 patients (24%) in the pediatric age group (<13years). Only 51 patients (7.9% of all cases) were above the age of 60 years. Out of a total of 640 cases, 378 were females (59.1%) and 268 were males (40.9%). The male: female ratio was 1.4:1. The cervical group of lymph nodes was most commonly involved as seen in 259 patients (40.4% of all cases). This was followed by submandibular lymphadenopathy in 100 patients (15.7%). Submental involvement was seen in 67 patients while 64 cases presented with supraclavicular lymphadenopathy (10% of the total). Other lymph node groups involved were the axillary region in 52 cases (8.2%), infraclavicular lymphadenopathy in 60 patients, and inguinal lymph nodes consisting of 38 cases (5.9 %). Persistent cough was the most common associated presenting symptom, apart from lymphadenopathy, noted in 439 patients (68.5% of the total). Other

associated findings were fever of variable intensity reported by 369 patients (57.6%), weight loss (51.7% cases), and loss of appetite (40.0% cases). Four main cytomorphological patterns were recognized. The most common pattern was that of well-formed epithelioid cell granulomas with necrosis, seen in 197 cases (30.7% of all smears). This was characterized by epithelioid cell granulomas without necrosis (164 smears-25.6%). Pattern 3 showed necrosis without epithelioid cell granulomas, with neutrophilic infiltrates. This was observed in 148 (23.1%) aspirates. Pattern 4 was the least commonly observed smear pattern with, poorly formed granulomas or small lymphohistiocytic clusters, seen in 131 smears (20.4%).

Overall AFB positivity was found to be 40.3% in this study. Smears showing only necrosis without epithelioid cell granuloma showed maximum AFB positivity of 91.8%. followed by smears showing epithelioid cell granuloma with necrosis (52.7%). Poorly formed granuloma/small histiocytic clusters without necrosis showed the least positivity of 3.8%.

GeneXpert has an overall positivity rate of 84% with a maximum detection rate was seen in samples that showed epithelioid cell granuloma with necrosis (95.9%) on fine-needle aspirate followed by the pattern showing necrosis without epithelioid cell granuloma with neutrophilic infiltrates (95.2%).

DISCUSSION

The adult age group including patients between the age of 20 to 59 years constituted the largest group affected by tubercular lymphadenopathy with a total of 359 cases (56.0%). The pediatric age group was the second most affected group with 24% of the total cases. The result of this study was comparable to the study done by Muluye et al., (7) who observed maximum cases in the adult age group with 54.6% (25-60 years). A study was done by Singh et al., (8) Jasim et al.,(9) also made similar observations.

Out of 640 cases in the present study, females were more frequently diagnosed as suffering from tubercular lymphadenopathy (378 cases, 59.1% of all) with a ratio of female to male as 1.4:1. Similar observations were made by Khan et al (10) with the female: male ratio being 1.22:1. Mohapatra et al.,(11) again found a female preponderance with the female: male ratio being 2:1. One of the reasons for female predominance in most of these studies could be due to the poorer nutritional status of females from low socio-economic backgrounds, leading to higher susceptibility, in addition to other factors.

Although a combination of symptoms and signs were evident, fever and cough were noted to be the most frequent associated presenting complaints in most cases. Weight loss and loss of appetite were other accompanying features. The most common clinical finding of the present study was found to be persistent cough (68.5%) which was comparable with the study done by Pahwa et al., (11) and Gautam et al., (12). Similarly, other common findings like fever (57.6%) and loss of appetite (40.0%) in the present study were also in agreement with the above studies. However, in a similar study done by Khan et al., (10) found weight loss (15.7%) to be the most common clinical feature in patients with tubercular lymphadenopathy.

The most common cytomorphological pattern observed in the present study was epithelioid cell granuloma

with necrosis (30.7%) which was comparable with the study done by Shirish et al., (13) Chand et al.,(14) Balaji et al.,(15).

In the present study, ZN stain was able to detect acid-fast bacilli in 258 smears out of 640 cases suspected of tuberculous lymphadenitis on cytology with an overall positivity of 40.3%. Balaji et al., (15) in a similar study on 135 cases with tubercular lymphadenitis.

reported that Ziehl Neelsen staining for Acid Fast Bacilli (AFB) was positive in 32.5% of cases of TB Lymphadenitis. Lakhey et al., (16) also evaluated the role of the Ziehl-Neelsen stain in diagnosing tubercular lymphadenopathy on FNAC. Acid-fast bacilli were found in 58.1% of cases.

The difference in the rate of AFB detection by ZN staining in various studies could be attributed to the varying cytomorphological patterns, quality of sampling, and staining along with variation in time dedicated to the screening of the slides.

GeneXpert Assay showed an overall positivity of 84% in the present study. Similar observations were also shown in a similar study done by Denkinger et al., (17) (81.2%), Ligthelm et al., (18) (96.7%).

CONCLUSION

Fine-needle aspiration (FNA) cytology is minimally invasive and cost-effective. It is an outpatient diagnostic procedure, which easily lends itself to supportive tests such as staining for acid-fast bacilli, culture, and more recently, molecular testing by polymerase chain reaction-based systems. Nevertheless, the sensitivity and specificity are variable, concerning the diagnosis of tubercular lymphadenopathy. The diagnosis is rendered difficult in those cases where smears lack definitive features such as those showing poorly formed granulomas, or in cases of early tubercular lymphadenopathy with small lymphohistiocytic clusters or neutrophilic infiltrates. Nontuberculous

mycobacterial infection is another possibility that must be taken into consideration especially in the evaluation of immunocompromised individuals. Aspirates from such nodes may cause diagnostic dilemmas due to the absence of specific features of tubercular lymphadenopathy. The Ziehl-Neelsen (ZN) staining technique for acid-fast bacilli (AFB) is a simple, cheap, and rapid method. However, it has variable positivity as in the present study found to be 40.3%. Therefore, it cannot be relied upon to confirm or exclude a diagnosis of tuberculosis with certainty. New diagnostic molecular tests like GeneXpert MTB/RIF Assay help in the rapid detection of mycobacterium tuberculosis (less than two hours) even in smear-negative cases and have an overall high detection rate (84% in the present study). Thus, in a developing country like India with a high prevalence rate of tuberculosis, FNAC coupled with Z.N. staining and GeneXpert should be the first-line investigation in cases with lymphadenopathy. After cytopathological diagnosis, the decision regarding AFB staining and molecular testing with GeneXpert MTB/RIF Assay should be taken regarding the certainty of diagnosis.

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Ethical Approval: Approved

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