

Comparative Study of Fine Needle Aspiration Cytology, Acid Fast Bacilli Staining and Cartridge Based Nucleic Acid Amplification test in the Diagnosis of Extrapulmonary Tuberculosis

Nilima Chaudhari¹, Chandni Patel²

¹Assistant Professor, Department of Pathology, NAMO Medical Education and Research Institute, Silvassa, Dadra and Nagar Haveli, India

²Assistant Professor, Department of Pathology, NAMO Medical Education and Research Institute, Silvassa, Dadra and Nagar Haveli, India

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Corresponding author: Chandni Patel

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Abstract

Introduction: In underdeveloped nations such as India, tuberculous lymphadenitis is one of the most prevalent OPD presentations. However, anti-tuberculous medication cannot be supplied solely on the basis of clinical suspicion. Using cytomorphology with acid-fast labelling to diagnose these instances and cultures is a beneficial technique. The purpose of the study was to examine the utility and limitations of fine needle aspiration cytology, as well as the varied cytomorphological presentations of Ziehl-Neelsen staining in tuberculous lymphadenitis, in correlation with the cartridge-based nucleic acid amplification test (CBNAAT) MTB.

Materials and Methods: The study was conducted for 2 years from January 2020 to December 2021 with 142 cases at a tertiary care centre, Shri Vinoba Bhave Civil Hospital, Silvassa, Dadra and Nagar Haveli. The patients with clinically suspected tuberculous lymphadenopathy were selected.

Results: AFB positivity was 30%. Epithelioid cell granulomas with caseous necrosis were the most common cytological picture and cases showing necrosis had highest AFB positivity. Posterior triangle was the most common site of involvement.

Conclusion: FNAC is a straightforward, safe, cost-effective, and practical method for diagnosing TB lymphadenitis. A comparison study of cytomorphological patterns, ZN staining for AFB, and the CBNAAT test for bacterial analysis can boost the diagnostic yield and diagnostic precision. Due to the absence of epithelioid granuloma or caseation necrosis in some cases of acute suppurative lymphadenitis, CBNAAT was a highly effective treatment strategy. CBNAAT's limitations include its inability to detect additional lymph node-associated diseases.

Keywords: AFB by ZN stain, Cartridge Based Nucleic Acid Amplification Test (CB-NAAT), Extra Pulmonary Tuberculosis (EPTB), Fine needle aspiration cytology (FNAC),.

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Introduction

In ancient times, it was believed that diseases resulted from the labour of an evil spirit, the wrath of hostile gods, or the activity of supernatural powers. During the evolution of the medical sciences, pioneers such as Louis Pasteur and Robert Koch independently realised that diseases were caused by pathogens that elicited a specific reaction from the body. Granulomatous response is one such response. Granulomatous lymphadenitis is characterised by the appearance of several diseases, such as mycotic, viral, and bacterial infections, such as tuberculosis, leprosy, syphilis, Sarcoidosis, and toxoplasmosis, as well as a secondary response in lymph nodes draining carcinomas or lymphomas [1]. In developing nations, TB poses a substantial hazard to public health. Per day in India, one thousand people die from tuberculosis, which translates to one death every minute [2,3,4]. Outside of the lungs, the prevalence of tuberculosis is increasing globally. It is a protean disease, meaning it can affect virtually every organ in the body [5]. It is hypothesised that immunosuppression caused by HIV is the driving reason behind the global increase.

The cervical region is the area of the body most usually affected by extrapulmonary mycobacterial disease [3,6,7]. The most prevalent form of extrapulmonary mycobacterial illness is involvement of peripheral lymph nodes. Fine needle aspiration cytology (FNAC) is a less expensive, more efficient, and less hazardous alternative to histopathology for the detection of tuberculosis. In addition to being patient-friendly, this approach provides an accurate examination of cytomorphological properties. The presence of an epithelioid granuloma is the determining factor in diagnosing tuberculosis. Even in the absence of epithelioid cell granulomas, the aetiology can be conclusively proven by exhibiting

AFB in FNAC smears either directly or through culture [2,8].

The objectives of the present study were to determine the diagnostic value of FNAC in tuberculous lymphadenitis and the utility of Ziehl-Neelsen staining and CBNAAT for MTB, to determine the prevalence of tuberculosis in clinically suspected patients of tuberculous lymphadenitis, and to analyse the cytomorphological features observed in aspirates of tuberculous lymphadenitis and correlate them with acid fast bacilli positivity. On Ziehl Neelson staining and CBNAAT for MTB

Aim and Objective:

To assess the efficacy of FNAC positivity compared with ZN staining and CBNAAT test in diagnosing tuberculous lymphadenitis.

Material and Method:

The present retrospective 2 years study was conducted at tertiary care hospital of Shri vinoba bhav civil hospital, Silvassa, Dadra and Nagar Haveli from January 2020 to December 2021. Total 142 cases with lymphnode swelling underwent FNAC, ZN staining and CBNAAT test. Aspiration were done by using 10/5ml syringe and 22/23 gauge needles. Methanol fixed smears were made and stained with Hematoxylin & Eosin and PAP stain for examined of epithelioid granuloma with or without necrosis, Two air dry smear was stained with ZN stain to look for acid fast bacilli and remaining aspirated material was used for CB-NAAT testing for detection tuberculosis bacteria with results was reported as positive or negative. The sites of Tuberculosis lymphnode commonly include cervical lymphnodes, Axillary lymphnodes and Inguinal lymphnode.

Results

A total of 142 clinically suspected patients of tuberculous lymphadenitis are included in the present study. The period lasted for

one year from January 2020 to December 2021. Out of 142 patients, presented only with variable duration cervical lymphadenopathy. Out of 142 cases, majority of the patients between 15 to 30 year age group (79,55.6%) followed by 31 to 45 year age group (36, 25%). Out of 142 samples, 84 (59.15%) patients were female and 58 patients were males (40.84%)

showing female preponderance. Cervical lymphnode (123, 86.6%) was most commonly involved followed by axillary lymph node (14, 9.9%). Purulent material was found in 48 (33.8%) aspiration samples and blood mixed material in 41 samples (28.9%) and blood mixed purulent material was found in 53 samples (37.3%).

Table 1: Basic characteristics of the study population

Characteristics	No. of Cases (%)
Age Group (years)	
15-30 years	79 (55.6%)
31-45 years	36 (25.3%)
45-60 years	20 (14.1%)
> 60 Years	7 (4.9%)
Gender	
Female	84 (59.1%)
Male	58 (40.8%)
Site	
Cervical lymph node	123 (86.6%)
Axillary Lymph Node	14 (9.9%)
Inguinal lymph node	2 (1.4%)
Soft tissue swellings	3 (2.1%)
Aspiration Material	
Only purulent material	48 (33.8%)
Blood mixed material	41 (28.9%)
Blood mixed purulent material	53 (37.3%)

Epithelioid cell granulomas with caseous necrosis was observed in 52 samples on microscopic examination. Out of 52 samples, it was detected in 47 samples (90.4%) on AFB and 50 samples (87.7%) on CBNAAT.

Table 2: Summary of AFB positive and negative cases by CB-NAAT method, ZN staining method along with correlation to FNAC findings

Microscopic examination	AFB positive by ZN staining		CBNAAT positive cases	
	Positive	Negative	Positive	Negative
Epithelioid cell granulomas without caseous necrosis (n=43)	12 (27.9%)	31 (72.1%)	17 (39.5%)	26 (61.5%)
Epithelioid cell granulomas with caseous necrosis(n=52)	47 (90.4%)	5 (9.6%)	50 (96.6%)	2 (3.4%)
Necrosis with neutrophils (n=33)	29 (87.9%)	4 (12.1%)	32 (90.9%)	1 (9.1%)
Total	88	40	99	29

Table 3: Comparison of CB-NAAT result with FNA findings suggestive of Tubercular etiology

FNA	CB-NAAT		
	Positive cases (n-105)	Negative cases (n-224)	Chi square, p value
Positive cases (n-128)	99	29	X ² – 198.98, p < 0.001
Negative cases (n-201)	6	195	

Statistically significant difference was seen between CBNAAT and FNAC at p value <0.001

Table 4: Comparison of CB-NAAT result with AFB (n=128)

AFB by ZN stain	CB-NAAT		
	Positive cases (n-99)	Negative cases (n-29)	Chi square, p value
Positive cases (n-89)	88	1	X ² – 77.29, p < 0.001
Negative cases (n-39)	11	28	

Statistically significant difference was seen between CBNAAT and AFB at p value <0.001

Table 5: Predictive validity of FNAC and AFB compared to CB NAAT

Parameter	FNAC	AFB by ZN staining
Sensitivity	94.3% (87.9% to 97.9%)	88.9% (81.0% to 94.35)
Specificity	87.1% (81.9% to 91.2%)	96.6% (82.2% to 99.9%)
PPV	18.3% (13.7% to 24.0%)	98.9% (92.7% to 99.8%)
NPV	99.8% (99.6% to 99.9%)	71.8% (59.3% to 81.7%)
Diagnostic accuracy	87.3% (83.2% to 90.7%)	90.6% (84.2% to 95.1%)

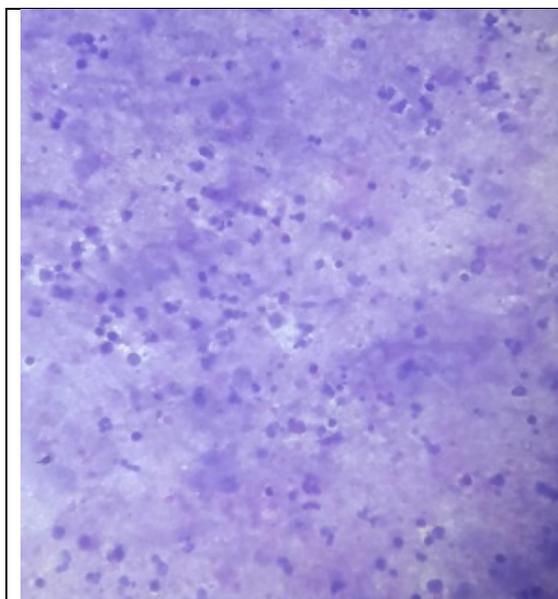


Figure 1: Suppurative lesion -Smear shows sheets of polymorphs [H&E stain-40X]

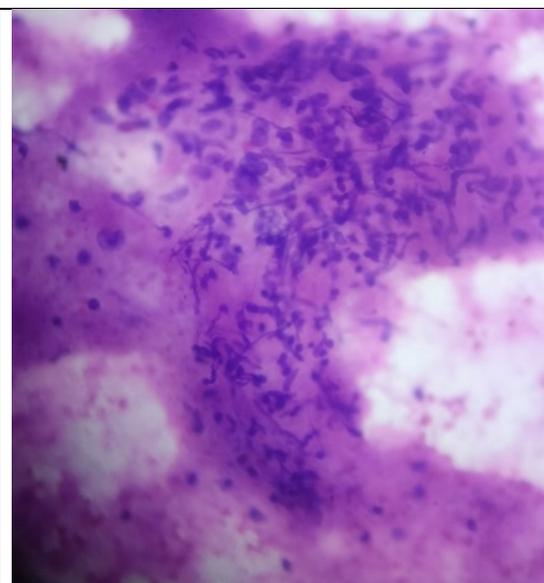


Figure 2: Epithelioid granuloma with necrosis-Smear shows granuloma composed of epithelioid cells with necrotic background [H&E stain-40X]

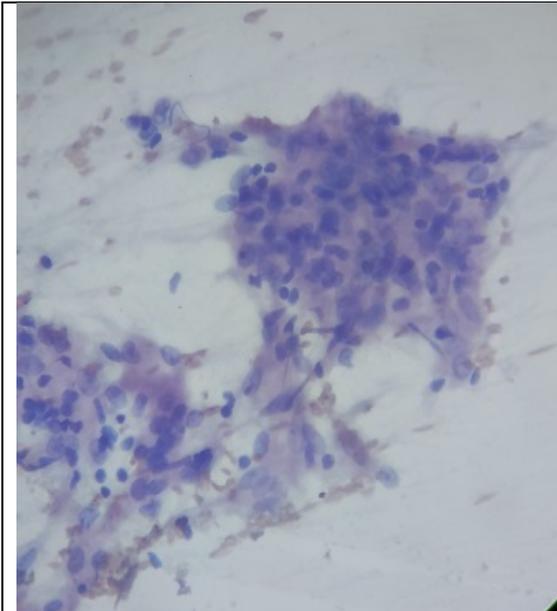


Figure 3: Epithelioid granuloma without necrosis-Smear shows a granuloma composed of epithelioid cells with no necrosis in the background [Pap stain 40X]



Figure 4: Acid Fast bacilli- Smear shows bacilli scattered among few polymorphs [Z-N stain-100X]

Sensitivity and NPV are seen too high in FNAC compared to AFB, while diagnostic accuracy, specificity, PPV was higher in AFB staining than FNAC

Discussion

In the present study, non-culture diagnostic procedures, such as fine needle aspirates, were utilised to confirm the diagnosis of tuberculous lymphadenitis. Faster results allow us to give earlier recuperation, which is crucial for preventing the disease's spread. In this study, there was a little female preponderance among patients of tuberculous lymphadenitis with clinical suspicion. This study is comparable to research undertaken at the tertiary hospitals VMMC and Safdarjung Hospital in New Delhi, which likewise revealed a female preponderance. [9]

Similar to the study conducted by Dhawan I et al. [9] revealed that TB lymphadenitis is most prevalent in the age range of 15 to 30 years, which includes the productive age group.

In this study, the posterior triangle of cervical lymph nodes were most frequently affected by lymphadenopathy, followed by the anterior triangle of cervical lymph nodes, supraclavicular lymph nodes, and submandibular lymph nodes. This observation parallels the research conducted by Bibhuti Das et al. [10]

In our study, cervical lymphadenopathy is the most common presentation in 123 (86.6%) and inguinal lymphnodes 2 (1.4%) were least commonly involved, similar to the Mailamani S et al. [11] study and Siddegowda MS et al. [12] in which cervical groups involved was 97.2% and 67% respectively. These might be due to organism usually gains access to the cervical lymph nodes through the tonsillar lymphoid tissue.

In this investigation, only 20 samples were purulent, whereas the rest 122 samples were minimally fluid and/or blood-free. Twelve of twenty purulent samples tested positive, whereas 82 of the remaining non-purulent (minimum fluid) samples were positive. The molecular investigation has

been limited to a small number of samples due to the difficulty of obtaining a sufficient sample outside of fluctuating patterns. In the present investigation, 44 samples out of 142 tested positive for AFB using the Ziehl-Neelsen method.

Cytomorphological FNA cytology demonstrating epithelioid cells, granulomas with or without multinucleated giant cells, and caseation necrosis were diagnostic criteria for tuberculosis. Cytology demonstrating necrosis only/non-caseating granulomas/acute suppurative lymphadenitis in the absence of AFB was suggestive for tuberculosis. In our study, there was a correlation between necrosis and AFB-positive instances. Cytomorphological pictures of tuberculous lymphadenitis was described by various author divided in different pattern or category. In our study microscopic finding were divided in 3 categories. Most common pattern was Epithelioid cell granulomas with caseous necrosis (36.6%). This findings is well correlated with study done by Mailamani S et al. [11] study in which 48.1% cases shows these Epithelioid cell granulomas with caseous necrosis type of pattern.

In our study, maximum number of AFB positivity is observed in pattern 2-Epithelioid cell granulomas with caseous necrosis in 94.2% (47/52) caases followed by in pattern 3 necrosis with inflammation 97% cases (29/33) which is similar to study done by Mailamani S et al. [11] study in which positive cases by AFB in pattern C as necrosis without epithelioid granulomas (77.1%) followed by pattern B Epithelioid cell granulomas with caseous necrosis (60.8%). We have slightly higher number of case because of many migrant and industrial areas. We have taken CBNAAT as the standard for comparing validity of FNAC and AFB positivity.

Comparing the validity of FNAC and AFB positive, we have used the CB-NAAT as the benchmark. Therefore, the predictive

validity of FNAC was 94.3% sensitive, 87.9% specific, 18.3% PPV, 99.8% NPV, and diagnostic accuracy was 87.3%. In a study conducted by Tadesse et al., the diagnostic accuracy of cytology was assessed in comparison to a composite reference standard with sensitivity of 80%, specificity of 57.8%, PPV of 79.1%, and NPV of 59.0%. In a study conducted by Siddegowda MS et al. [12], the predictive validity of FNAC compared to CB-NAAT had a sensitivity of 85.7%, a specificity of 73.8%, a false positive rate of 26.1%, a false negative rate of 14.2%, PPV of 63.8%, NPV of 90.5%, and a diagnostic accuracy of 78%. The predictive validity of AFB in the CB-NAAT result is 88.9% sensitivity, 96.6% specificity, 98.9% PPV, 71.8% NPV, and 90.6% diagnostic accuracy. Siddegowda MS et al. [12] study determined that the predictive validity of AFB in the CB-NAAT has a sensitivity of 37.1%, specificity of 100%, false positive rate of 0%, false negative rate of 62.8%, PPV of 100%, NPV of 74.7%, and diagnostic accuracy of 78%. [13]

The formation of granuloma is dependent on immune system of host. In immunocompromised immune system is not efficient to result in granuloma formation Hence AFB positivity is observed without granuloma formation other immunodeficient comorbidities should be considered in mind.

Conclusion

In terms of diagnostic yield, the AFB has an advantage over cytology for diagnosing tuberculous lymphadenitis. On microscopic examination, epithelioid cell granulomas with caseous necrosis were most frequently identified, as well as positive for AFB and CBNAAT. The sensitivity of FNAC is better than that of AFB, although the diagnostic accuracy of AFB staining was greater than that of FNAC. CB-NAAT is a rapid, simple test for early diagnosis of EPTB because of high specificity and PPV. It can also detect the cases missed by FNAC and ZN

techniques. CB-NAAT combined with clinical data, FNAC and ZN staining will be effective in the diagnosing EPTB.

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