https://africanjournalofbiomedicalresearch.com/index.php/AJBR
Afr. J. Biomed. Res. Vol. 27(3s) (September2024) 1312 - 1318
Research Article

Comparative Analysis Of Cytomorphological Patterns And CBNAAT Results In Diagnosing Extra-Pulmonary Tuberculosis: A Retrospective Study

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Abstract:
Background: Extra-pulmonary tuberculosis (EPTB) offers diagnostic problems due to its diverse clinical presentations and low bacterial burden. While the Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) provides high diagnostic accuracy and quick findings, Fine Needle Aspiration Cytology (FNAC) remains a low-cost, non-invasive diagnostic approach. The purpose of this study is to evaluate cytomorphological patterns in FNAC of CBNAAT-positive EPTB cases and compare them to CBNAAT results.
Methods: This retrospective analysis was carried out in Government Medical College Karur from June 2021 to May 2024. We collected CBNAAT-positive EPTB cases and examined their FNAC slides. Clinical information, such as age, gender, and clinical presentation, were obtained. FNAC slides were examined for cytomorphological features including granuloma, caseous necrosis, multinucleated giant cells, and fibroblasts. CBNAAT results were divided into four categories: very low, low, medium, and high bacterial burdens. The sensitivity of cytomorphological patterns was also assessed.
Results: Out of 34 CBNAAT-positive EPTB cases, the majority were 51-60 years old, with a small female predominance. Lymph nodes were the most prevalent site (61.7%). CBNAAT discovered MTB at four levels: very low (41.1%), low (35.3%), medium (20.5%), and high (2.9%), with one case demonstrating Rifampicin resistance. Cytomorphological patterns revealed granuloma with caseous necrosis in 44.1% of patients, with lymph nodes being more sensitive (65%) to granuloma with caseation. High MTB values frequently lacked identifiable cytomorphological characteristics, implying immunosuppression.
Conclusion: FNAC is a useful first diagnostic technique for EPTB, especially in lymph nodes, where it is highly sensitive when paired with caseation. However, the presence of a high MTB load in the absence of normal cytomorphological traits suggests that more research into immunosuppressive consequences is needed. CBNAAT is critical for the correct and timely diagnosis of EPTB.

Keywords: Extra-pulmonary tuberculosis, Fine Needle Aspiration Cytology (FNAC), Cartridge-Based Nucleic Acid Amplification Test (CBNAAT), cytomorphological patterns, diagnostic accuracy, Rifampicin resistance

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Received; 25/08/2024 Accepted: 26/09/2024

DOI: https://doi.org/10.53555/AJBR.v27i3S.2276

Introduction

Extra-pulmonary tuberculosis (EPTB) is a significant public health concern that frequently complicates tuberculosis (TB) diagnosis and treatment. Unlike pulmonary tuberculosis, which primarily affects the lungs and may be identified by sputum smear microscopy, culture, or molecular approaches, EPTB affects other organ systems such as lymph nodes, pleura, bones, and the central nervous system. [1] EPTB diagnosis is intrinsically more challenging because to its diverse clinical manifestations and the often limited availability of acceptable specimens for direct testing. [2] Diagnosis of EPTB is formidable task due to the variable clinical presentation and presence of low bacterial load in the sample obtained. [3]Cartridge Based Nucleic Acid Amplification Test (CBNAAT) is a latest automated cartridge-based molecular diagnostic test with highest diagnostic accuracy which gives results within 2 hours and has been endorsed by WHO as an initial diagnostic test in suspected cases. [4] It has added advantage of identifying the rifampicin resistance and semiquantitative estimate of the concentration of the bacilli. [5] Globally the use of rapid molecular tests is increasing.CBNAAT-high sensitivity and less time taking procedure makes it an excellent tool for timely diagnosis of Extra-pulmonary tuberculosis. Fine Needle Aspiration Cytology (FNAC) remains as a simple cost effective, easy and less invasive procedure in the diagnosis of extrapulmonary tuberculosis especially in resource limited health facilities with high diagnostic accuracy. [6, 7] However, diagnosis of EPTB solely based on cytomorphology alone is debatable. Given these factors, a comparison of cytomorphological patterns and CBNAAT results may provide useful information about their distinct functions and diagnostic usefulness in EPTB. This retrospective study will assess the effectiveness of FNAC and CBNAAT in identifying EPTB, with the goal of improving diagnostic techniques and patient outcomes.

Materials and Methods

This is a retrospective study carried out in the Department of Pathology and Microbiology, Government Medical College, Karur, from June 2021 to May 2024. The line-up of CBNAAT-positive extrapulmonary tuberculosis cases was obtained from the records. FNAC slides from CBNAAT-positive EPTB cases were collected. The clinical data includes age , gender and clinical presentation was obtained from the NIKSHAY site. FNAC slides were also examined for several cytomorphological patterns such as the existence of granuloma, caseous necrosis, the nature of the background, the presence of multinucleated giant cells and the presence of fibroblasts. Comparative examination of cytomorphological patterns with CBNAAT reports aids in confirming the diagnosis and highlighting the correlation.

According to standard operating procedure the sampling reagent was added at 2:1 ratio to the sample and kept for 15 min at room temperature with intermittent shaking. 3 mL of this treated sample was transferred to the cartridge and the cartridge was inserted in the module of CBNAAT machine. An automatic process completed the remaining assay steps and the results were displayed on the monitor attached to Gene Xpert after 1 h and 50 min. Detection of mycobacteria and rifampicin resistance was carried-out in the same setting.The CB-NAAT system detects DNA sequences specific for *Mycobacterium tuberculosis* (MTB) complex and rifampicin resistance by Real Time Polymerase Chain Reaction (PCR).

It concentrates *Mycobacterium tuberculosis* bacilli from the samples, isolates genomic material from the captured bacteria by sonication and subsequently amplifies the genomic DNA by PCR. The process identifies clinically relevant rifampicin resistance-inducing mutations in the Beta subunit of RNApolymerase (rpoB) gene in the *Mycobacterium tuberculosis* genome in a real-time format using fluorescent probes called molecular beacons.

The limit of Detection of CBNAAT is (LOD ~ 130 CFU/ml), it is more sensitive (detects cases even when the bacillary load is low). Patients on an anti-TB regimen can still have positive results due to killed bacilli in the specimen and hence CB-NAAT cannot be used for follow-up.

Inclusion Criteria:

- Extrapulmonary tuberculosis cases with positive CBNAAT results.
- Clinical Data Availability
- Histological and Clinical Diagnosis

Exclusion Criteria:

- Suspected Extrapulmonary tuberculosis cases with negative CBNAAT results
- Pulmonary tuberculosis cases
- Inadequate FNAC Specimens
- Incomplete Clinical Data

Results

A total of 34 CBNAAT-positive EPTB cases were analyzed. The majority of EPTB cases were reported in the 51-60 age range, accounting for 23.5% of all cases.

This was followed by the 41-50 age group, which had 20.5% of the cases. The age groups 21-30 and 31-40 contributed evenly, accounting for 17.6% of the total cases. The younger age groups (<10 years and 11-20 years) had much fewer cases, with rates of 5.8% and 2.9%, respectively. The age group older than 60 years accounted for 11.7% of the cases. This distribution shows a higher prevalence of EPTB in middle-aged to older persons, implying that the condition is more common in this age range in the studied population. The distribution of these cases according to age is detailed in Table 1. The 34 CBNAAT-positive EPTB patients studied showed a slight female predominance. Specifically, 18 instances (53%) were female and 16 cases (47%) were male. This suggests that EPTB occurred somewhat more frequently in females than in males in this study sample (Table.2).

Table1: Distribution of EPTB according to age

Age group	No of cases	Percentage (%)
<10	2	5.8
11-20	1	2.9
21-30	6	17.6
31-40	6	17.6
41-50	7	20.5
51-60	8	23.5
>60	4	11.7

Table 2: Distribution of EPTB according to gender

Gender	No of cases	Percentage (%)
Male	16	47
Female	18	53

Table 3 summarizes the site-specific distribution of EPTB patients. CervicalLymph nodes were the most commonly afflicted site, accounting for 61.7% of cases, followed by axillary lymph nodes (6.0%) and inguinal lymph nodes (3.0%). This implies that lymph nodes are predominantly involved in EPTB cases. The second most prevalent place was pleural fluid, which accounted for 32.3% of cases. Ascitic and synovial fluids both accounted for 3.0% of cases. Furthermore, one individual (5.8%) had an arm abscess. These findings show a strong prevalence of lymph node involvement in EPTB, with considerable but less common occurrences in bodily fluids and abscesses, demonstrating the disease's wide range of manifestations.

Table: 3 Distribution of EPTB according to site

	Site	No of cases	Percentage (%)
Lymph nodes	cervical	18	61.7
	Axillary	2	
	Inguinal	1	
Body Fluids	Pleural fluid	9	32.3
	Ascitic fluid	1	
	Synovial fluid	1	
Abscess	Arm	1	5.8
	Psoas	1	

Table 4 depicts the distribution of EPTB cases based on cytomorphological patterns seen in FNAC specimens. The most common cytomorphological pattern was granuloma with caseous necrosis, which occurred in 44.1% of cases. This was followed by patients with just caseous necrosis, accounting for 14.7%. Only 2.9% of cases had granulomas, whereas 5.8% had only acute inflammatory cells. Additionally, lymphocytic effusion was found in 32.3% of patients. These data show that granuloma with caseous necrosis is the most common cytomorphological feature in EPTB, while caseous necrosis alone and lymphocytic effusion are also seen often.

Table 4 Distribution of EPTB according to cytomorphological pattens

Cytomorphological patterns	Granuloma with caseous necrosis	Only granuloma	Only caseous necrosis	Only acute inflammatory cells	Lymphocytic effusion
No of cases	15	1	5	2	11
Percentage%	44.11	2.94	14.7	5.8	32.3

Cases with MTB very low, MTB low and MTB medium results showed cytomorphological patterns of epithelioid granuloma (Fig 1), caseous necrosis(Fig 2) and background acute inflammatory cells (Fig3) in most of the cases. Table 5 shows the relationship between cytomorphological patterns observed in FNAC and CBNAAT results, which are classified by bacterial burden as shown by CT values. Granuloma appeared in 42.8% of instances with a very low bacterial load, caseous necrosis in 50%, and background acute inflammation in 57.1%. Giant cells and fibroblasts were less common, appearing in 7% and 21.4% of cases, respectively. In instances with a low bacterial burden, granuloma (50%), caseous necrosis (75%), and acute inflammation (75%) were more common, while large cells and fibroblasts were detected in 8% and 50% of cases, respectively. Medium bacterial load cases similarly included granuloma (57.1%) and caseous necrosis (57.1%), but with less acute inflammation (28.5%) and no large cells, and fibroblasts were found in 28.5% of cases. Notably, in instances with a high bacterial load, no unique cytomorphological patterns were found, and just one case (2.9%) fell into this category. These findings suggest that, while granuloma, caseous necrosis, and acute inflammation are common in cases with lower bacterial loads, their presence decreases with higher bacterial loads, implying that high levels of infection may be associated with less distinct or altered cytomorphological features.

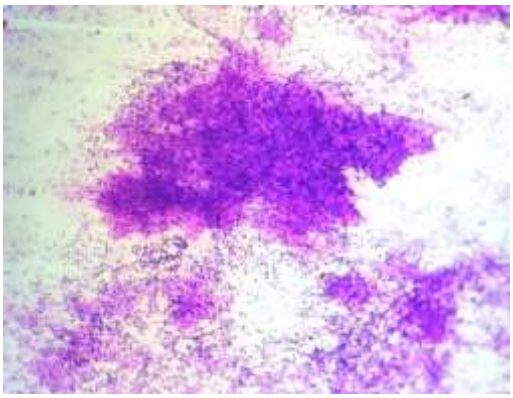

Fig 1: Epithelioid granuloma with caseous necrosis (H&E, 10x view)

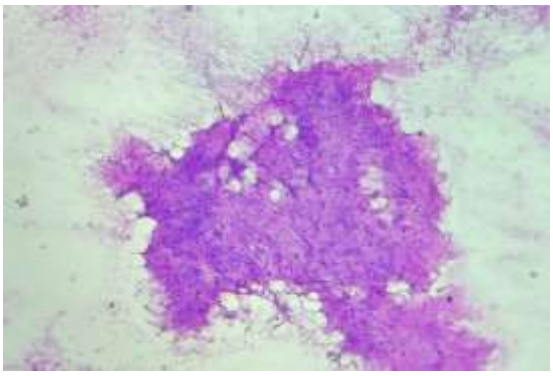

Fig 2: Caseous necrosis only (H&E, 10x view)

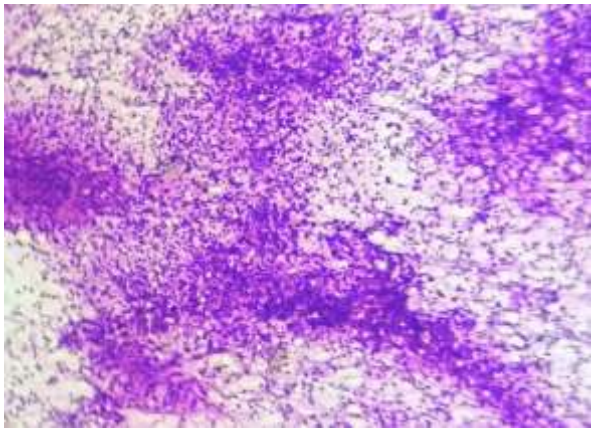

Fig 3: Epithelioid granuloma with background acute inflammation (H&E, 10x view)

Table 5: Correlation of cytomorphological patterns with CBNAAT Results

CBNAAT CT Value	Granuloma		Caseous necrosis		B/g Acute inflammation		Giant cells		Fibroblasts		Total	
	No	%	No	%	No	%	No	%	No	%	No	%
Very low	6	42.8	7	50	8	57.1	1	7	3	21.4	14	41.1
Low	6	50	9	75	9	75	1	8	6	50	12	35.3
Medium	4	57.1	4	57.1	2	28.5	0	0	2	28.5	7	20.5
High	0	0	0	0	0	0	0	0	0	0	1	2.9
Total	16	47.0	20	58.8	19	55.9	2	5.8	11	32.3	34	100

The sensitivity of cytomorphological patterns for identifying EPTB was assessed using caseation with granuloma. Overall, the sensitivity for detecting caseation with granuloma was 44%. However, concentrating solely on lymph node instances raised sensitivity to 65%. This suggests that caseation with granuloma is a more accurate diagnostic characteristic in lymph node EPTB. Furthermore, when evaluating instances with either caseation necrosis, granuloma, or both, the sensitivity increased dramatically to 91%. This shows that the combination of these characteristics serves as a highly sensitive diagnostic sign for EPTB, particularly when assessing lymph node involvement.

Discussion

A comparison of cytomorphological patterns in FNAC with CartridgeCBNAAT results for EPTB reveals numerous interesting findings. Our findings emphasize the diagnostic value of FNAC and its complimentary role to molecular testing, particularly in the presence of variable bacterial burdens. The common site of involvement in EPTB is lymph nodes, pleura and gastrointestinal tract, it also occurs in genitourinary system, central nervous system, bone and spine. [8] The gold standard diagnostic test for EPTB is culture with Lowenstein Jensen media, but it is time consuming. [9] CBNAAT has emerged as a rapid and accurate investigation for EPTB. [4] FNAC can be used as an initial investigation in EPTB because they obtained sample with single prick can be used for cytological examination, CBNAAT and Ziehl Nielson staining. This study is aimed to evaluate the usefulness of FNAC in the diagnosis of EPTB and to assess the variable cytomorphological patterns obtained in CBNAAT positive EPTB cases based on CBNAAT software generates the results from the measured fluorescence signals and embedded calculation algorithms. In addition to qualitative results, the software provides semi-quantitative results, which provide information about the bacterial load in the specimens tested. Semi-quantitative result interpretation is based on Cycle threshold (Ct) values of *Mycobacterium tuberculosis* specific targets. Cytological features may show epithelioid granulomas, caseous necrotic material, multinucleated giant cells, fibroblasts and acute inflammatory cells or combinations of these features. [10] T cell mediated host immune response to the bacteria results in the development of pathological lesions such as granulomas and caseous necrosis.Immunocompromised people do not form the characteristic granulomas and their macrophages contain many bacilli.

In our study, 34 CBNAAT positive EPTB cases were included. Majority of the patients (23.5%) were from the age group of 51-60 years. But Bezabih et al., [11] Lobo et al., [12] Majeed et al., [13] Patel et al.,[14] and Krishna et al.,[15] who reported the maximum prevalence of the disease between the ages of 11- 30 years.Our study showed a female preponderance which was in concordance with other studies Patel et al.,[16] Paliwal et al.,[17]Majeedet al.,[13] and Natraj et al.,[18] have shown female preponderance. But studies by of Gupta et al.,[19]Kivihiya- Nduggaet al.,[20] and Annam et al.,[21] have shown a male preponderance. The most common site of aspiration was lymph node (61.7 %) which correlates with the findings of Patel et al.,[16]Ahmadet al.,[22] Eagaet al.,[23] Bezabihet al.,[11], Majeed et al.,[13] and Krishna et al.,[15], who have also reported the cervical lymph node as the most common site.Our finding was in concordance with the all of the studies below that showed epithelioid cell granuloma with caseous necrosis as the most common cytomorphological pattern.

Study/ Ref	Granuloma with caseous necrosis (%)	Only granuloma (%)	Only caseous necrosis (%)	Only acute inflammatory cells (%)
Our study	44.11	2.94	14.7	5.8
Patel et al, 2015 [14]	35.61	-	23.92	-
Majeed et al, 2011 [13]	69	31	-	-
Ahmad et al, 2005 [14]	45.8	28.9	25.3	-
Bezabih et al, 2002 [11]	32.8	15.6	51.2	-
Gupta et al, 2002 [19]	50.35	32.14	14.65	-

Cases with MTB very low, lowand medium results showed cytomorphological patterns of epithelioid granuloma, caseous necrosis and background acute inflammatory cells in most of the cases. None of the cytomorphological pattern of tuberculosis werepresent in case with MTB high result.MTB high result indicates increased bacilli load in the lesion.The number of cases with high MTB result is only one case

in our study. This caseshowednone of the characteristic cytomorphological features such as granulomata and caseous necrosis.This can be explained on the fact that Granulomata and caseous necrosisoccur as result of host immune defence. So high bacterial load with absence of characteristic cytomorphological features indicates the immunosuppression. But the significance is of questionable as we have only one case of MTB high.Hence the association of high MTB result with absence of characteristic cytomorphological patternsuch as granuloma and caseous necrosis needs further study.

The total sensitivity of 44% for granuloma with caseation suggests that, while this pattern can be informative, it may not be present in all EPTB patients. The enhanced sensitivity to 65% in lymph nodes and 91% in the presence of caseation necrosis or granuloma emphasizes the need of combining several diagnostic techniques. FNAC should thus be viewed as a complimentary tool to CBNAAT, particularly in resource-constrained environments where FNAC provides a less intrusive and cost-effective diagnostic option. Our findings were in concordance with the following studies. Gouda K et al [(24)] reported the diagnostic efficiency of FNAC in diagnosing EPTB with sensitivity of 72.71%, Siddegowda et al [(25)] calculated the sensitivity of 85.7%, specificity of 73.8%, Tadesse et al [(26)] reported sensitivity of 80%, specificity of 57.8%. So, the presence of either granuloma or caseation or both is a sensitive tool in detecting EPTB especially in TB lymph node. Cytomorphology is not a sensitive tool in detecting TB effusion.

Conclusion

EPTB can be easily diagnosed based on the cytomorphological patterns. It is recommended to use FNAC as an initial investigation of choice for diagnosing EPTB. CBNAAT plays an important role in diagnosing EPTB in paucibacillary lesions and alsoto identify the rifampicin sensitivity.Addition of simple affordable rapid FNAC test with CBNAAT increases the diagnostic accuracy.Hence FNACcan be used as a sensitive tool especially in lymph node for diagnosing EPTBin resource limited health facilities.

Acknowledgement

The authors are thankful to all the staffs in the Department of Microbiology, Department of Pathology, Government Medical College Karur, TB DDHS, Karur District for their timely extraordinary help in conducting this study and writing this manuscript.

Conflict of interest

The authors declare no conflict of interest.

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