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Original Research Article

Comparative study of fine needle Aspiration Cytology, Acid Fast Bacilli staining and Cartridge Based Nucleic Acid Amplification test in the diagnosis of extrapulmonary tuberculosis

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ABSTRACT

Introduction: Tuberculosis (TB) is an important health problem in the developing countries. Extrapulmonary sites of TB commonly include lymph nodes, pleura, gastrointestinal system, genitourinary system, central nervous system, bones. Most of the EPTB cases present with lymphadenopathy and effusion. The conventional methods to diagnose EPTB includes fine needle aspiration cytology (FNAC) and demonstration of acid fast bacilli (AFB) using Zeihl- Neelson (ZN) staining.
Objectives: 1). To assess the efficacy of FNAC positivity in comparison to CB-NAAT in the diagnosis of EPTB. 2). To assess the efficacy of AFB positivity in comparison to CB-NAAT in the diagnosis of EPTB.
Materials and Methods: The present retrospective record based study was conducted at the Department of Pathology and CB-NAAT Centre, Mandya Institute of Medical Sciences (MIMS), Mandya, Karnataka. The study was started after obtaining approval from the Institutional Ethics Committee.
The records of suspected 100 EPTB patients who had under gone FNAC, ZN stain and CB-NAAT test for EPTB diagnosis during the study period of four months from March to June 2018 were analysed.
Results: A total of 100 subjects were included in the final analysis. In our study, youngest patient was 8 months and oldest was 75 years. Of the 100 cases, lymph node involvement accounted for 96 cases and soft tissue swellings accounted for 4 cases. The diagnosis of FNAC of 100 cases were reactive lymphoid hyperplasia, suppurative lesion, epithelioid granuloma without necrosis, epithelioid granuloma with necrosis, caseous necrosis without epithelioid cells and lymphoma and their proportion were 30%, 21%, 22%, 13%, 12% and 2% respectively. Out of 47 cases with FNAC findings favouring EPTB, 30 were CB-NAAT positive and 17 were CB-NAAT negative. Out of 53 cases with negative FNA findings, CB-NAAT was positive in 5 cases (14.2%). Only 13 cases were positive for AFB when compared to 30 CB-NAAT positive cases. Among the 35 CB-NAAT positive cases, 13 (37.1%) cases were AFB positive.
Conclusion: 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.

1. Introduction

Tuberculosis (TB) is an important health problem in the developing countries. While TB affecting lungs is the most common presentation, extrapulmonary TB (EPTB) also accounts for 25% of the clinical burden.[1]

Diagnosis of EPTB is difficult due to clinical presentation with vague symptoms and signs, mimicking other chronic clinical conditions including neoplastic and inflammatory disorders, which results in either a postponement or lack of treatment.[2] A high index of suspicion is required to clinch the diagnosis. Paucibacillary character of EPTB poses a big challenge in the diagnosis of EPTB and multiple diagnostic modalities are required to arrive at correct diagnosis.

Extrapulmonary sites of TB commonly include lymph nodes, pleura, gastrointestinal system, genitourinary sys-

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tem, central nervous system, bones/spine although any organ can be involved. Most of the EPTB cases present with lymphadenopathy and effusion.[3]

The conventional methods to diagnose EPTB includes fine needle aspiration cytology (FNAC) and demonstration of acid fast bacilli (AFB) using Zeihl- Neelson (ZN) staining.

There are few limitations of FNAC like subjectivity of the test, failure to differentiate between various granulomatous diseases and absence of granuloma in immunocompromised patients.

There are also limitations of diagnosing EPTB by ZN staining like less sensitivity. Positive smear requires more than 5,000 to 10,000 bacilli/ml and hence it has limited diagnostic value in majority of EPTB samples.

Cartridge Based-Nucleic Acid Amplification Test (CB-NAAT)/ Gene Xpert is a fully automated, real time hemi-nested polymerase chain reaction system of molecular technology which has been endorsed by World Health Organisation (WHO) as the most sensitive rapid test for TB diagnosis in paucibacillary samples.[4]CB-NAAT test has very good positive predictive value, lesser negative predictive value, high sensitivity and specificity.[2] In 2014, WHO has recommended CB-NAAT over conventional tests for non-respiratory specimens from patients suspected of EPTB.[5]

2. Objectives

1. To assess the efficacy of FNAC positivity in comparison to CB-NAAT in the diagnosis of EPTB.
2. To assess the efficacy of AFB positivity in comparison to CB-NAAT in the diagnosis of EPTB.

3. Materials and Methods

The present retrospective record based study was conducted at the Department of Pathology and CB-NAAT Centre, Mandya Institute of Medical Sciences (MIMS), Mandya, Karnataka. The study was started after obtaining approval from the Institutional Ethics Committee.

The records of suspected 100 EPTB patients who had under gone FNAC, ZN stain and CB-NAAT test for EPTB diagnosis during the study period of four months from March to June 2018 were analysed. Inclusion criteria included patients of both sexes and all age groups who were suspected to have EPTB and had undergone FNAC, ZN staining and CB-NAAT tests during the study period. Exclusion criteria included EPTB patients who were already on treatment and EPTB patients diagnosed by pleural, peritoneal and cerebrospinal fluid analysis.

In a suspected EPTB case, FNAC was done using 22 gauge needle with 10 ml syringe attached to it. FNAC was done on multiple sites to ensure adequacy of sample wherever required. Three smears were prepared for Haematoxylin and Eosin, May-Grunwald Giemsa and ZN staining. The residual material from the remaining aspirate was rinsed into 0.7 ml sterile phosphate-buffered saline, incubated at room temperature and subsequently processed for CB-NAAT testing.[6]

Detection of AFB was done by ZN staining. The FNAC smear was air dried, heat fixed and the smear was flooded with strong carbol fuchsin which was then heated and rinsed off in tap water. Slide was then flooded with 1% solution of hydrochloric acid in isopropyl alcohol to decolourize and washed in tap water for 8 minutes. Then the smear was stained with methylene blue for 10 to 15 seconds, rinsed in tap water and dipped in distilled water. The smear was air dried, mounted and viewed under microscope.[7]

The FNAC samples of suspected EPTB cases were simultaneously referred to CB-NAAT centre, MIMS, Mandya. Detection of TB by CB-NAAT was done by Xpert MTB/RIF assay, made by Cepheid-Sunnyvale-USA. The specimens were processed according to the Gene Xpert system operator manual given by Central TB division, Government of India, Guidance document for the use of CB-NAAT under Revised National Tuberculosis Control Programme (RNTCP). The instrument contains 4 cartridges to process 4 samples at each run. According to the standard operating procedure, the assay sample reagent (containing NAOH and isopropanol) was added in 2:1 ratio to the sample and kept for 15 minutes at room temperature with intermittent shaking. Three ml of this treated sample was then transferred to the cartridge and the cartridge was inserted into the module of CB-NAAT instrument. An automatic process completes the remaining assay steps and the results were displayed on the monitor of Gene Xpert after 1 hour and 50 minutes.

Xpert MTB/RIF cartridge is a disposable, single self-enclosed test unit in which all steps of CB-NAAT, i.e., sample processing, PCR amplification and detection are automated and integrated. The manual steps involved in the assay are adding the reagent and sample loading. The test procedure is made biosafe by tuberculocidal property of the assay's sample reagent.[8,9]

The FNAC criteria for diagnosis of tuberculosis are the presence of clusters of epithelioid cells with or without giant cells and/ or caseous necrosis.[10]

A minimum of one slide positive even for single AFB/100 fields is taken as positive for mycobacterium tuberculosis.[11]

The CB-NAAT result is displayed as positive or negative in the CB-NAAT software.

The FNAC and ZN staining data was recorded in FNAC register, Department of Pathology and CB-NAAT data was recorded in CB-NAAT register of the CB-NAAT Centre, MIMS, Mandya.

4. Results

Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency and proportion for categorical variables. CB-NAAT was considered as gold standard. FNA findings with AFB were considered as screening test. The sensitivity, specificity, predictive values and diagnostic accuracy of the screening test along with their 95% CI were calculated. The p value of < 0.05 was considered statistically significant. The IBM SPSS version 22 was used for statistical analysis.

Total of 100 subjects were included in the final analysis

The median age was 28 years in the study population. In our study, youngest patient was 8 months and oldest was 75 years.

Among the study population there were equal number of male and female participants (50 each)

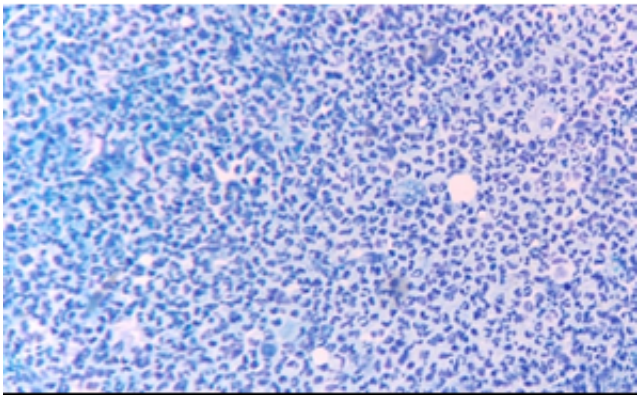

Fig. 1: Suppurativelesion -Smear shows sheets of polymorphs [MGG stain-40X]

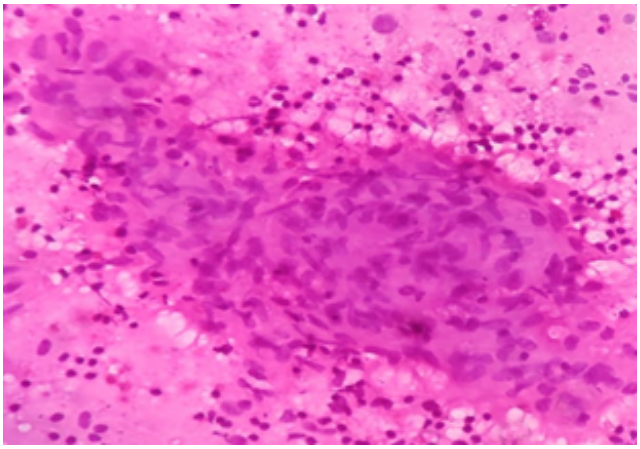

Fig. 2: Epithelioidgranuloma without necrosis-Smearshows a granuloma composed of epithelioid cells with no necrosis in thebackground [H&E stain 40X]

Of the 100 cases, lymph node involvement accounted for 96 cases and soft tissue swellings accounted for 4 cases.

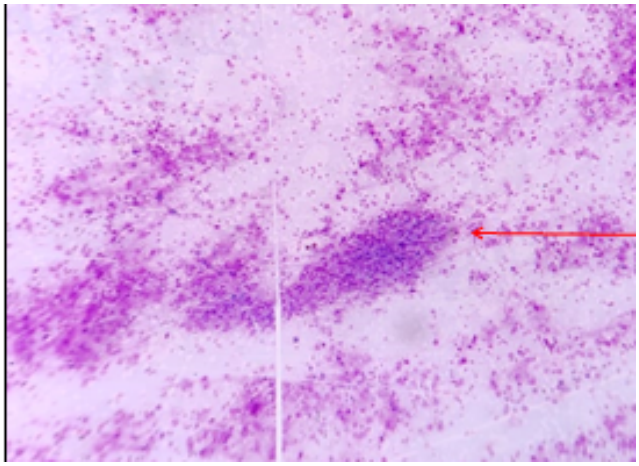

Fig. 3: Epithelioid granuloma withnecrosis-Smear shows granuloma composed of epithelioid cells with necrotic background [MGG stain- 10X]

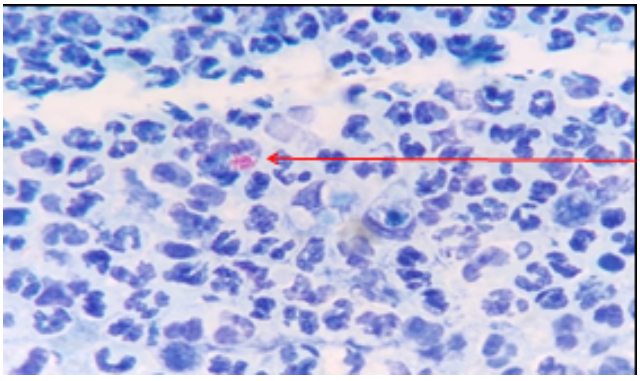

Fig. 4: AcidFast bacilli- Smear shows bacilliscattered among polymorphs [Z-N stain-100X]

Table 1: Descriptive analysis of gender in the study population (n=100)

Gender	Frequency	Percentage (%)
Male	50	50
Female	50	50

Among 96 cases involving lymph nodes, 84 cases were cervical lymph node involvement, 9 cases were axillary lymph node involvement and 3 cases were inguinal lymph node involvement. Among 4 cases of soft tissue swellings, 2 were parotid swellings and 2 were breast masses. (Table 2)

The diagnosis of FNAC of 100 cases were reactive lymphoid hyperplasia, suppurative lesion, epithelioid granuloma without necrosis, epithelioid granuloma with necrosis, caseous necrosis without epithelioid cells and lymphoma and their proportion were 30%, 21%, 22%, 13%, 12% and 2% respectively. (Table 3)

Out of 47 cases with FNAC findings favouring EPTB, 30 were CB-NAAT positive (Table 4) and 17 were CB-NAAT

Table 2: Descriptive analysis of site in the study population (n=100)

Site	Frequency	Percentage (%)
Cervical lymph node	84	84
Axillary Lymph Node	09	09
Inguinal lymph node	03	03
Other soft tissue swellings	04	04
Total	100	100

Table 3: Descriptive analysis of FNA features in the study population (n=100)

FNA features	Frequency	Percentage (%)
Reactive lymphoid hyperplasia	30	30
Suppurative lesion	21	21
Epithelioid granuloma without necrosis	22	22
Epithelioid granuloma with necrosis	13	13
Caseous necrosis without epithelioid cells	12	12
Lymphoma	2	02
Total	100	100

negative.

Table 4: Summary of CB-NAAT positive cases with FNAC findings suggestive of tubercular etiology (n=30)

FNAC diagnosis	No of cases with CB-NAAT positive	Percentage (%)
Epithelioid granuloma with caseous necrosis	11	36.7
Epithelioid granuloma without caseous necrosis	09	30.0
Caseous necrosis without epithelioid cells	10	33.3

Out of 53 cases with negative FNA findings, CB-NAAT was positive in 5 cases (14.2%) (Table 5). All these cases showed features of suppurative lesion in FNAC.

Compared to CB-NAAT, FNAC showed sensitivity of 85.7%, specificity of 73.8%, false positive rate of 26.1%, false negative rate of 14.2%, Positive predictive value (PPV) of 63.8%, Negative Predictive Value (NPV) 90.5% and diagnostic accuracy of 78% (Table 6)

Only 13 cases were positive for AFB when compared to 30 CB-NAAT positive cases. Among 13 AFB positive cases, epithelioid granuloma with caseous necrosis accounted for 8 cases, caseous necrosis without epithelioid cells and suppurative lesion accounted for 2 cases each and epithelioid granuloma without caseous necrosis accounted for 1 case.(Table 7)

Among the 35 CB-NAAT positive cases, 13 (37.1%) cases were AFB positive. (Table 8)

Predictive validity of AFB compared to CB-NAAT was 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%. (Table 9)

5. Discussion

CB-NAAT has been endorsed by World Health Organisation (WHO) as the most sensitive and rapid test for diagnosis of TB in paucibacillary samples. In our study youngest patient was 8 months and oldest was 75 years. Majority of the patients (35) were in the age group between 16 to 30 years with of age.This is in agreement with study by Tadeesse et al in 2015 where 83 out of 143 patients were in the age group between 16 to 30 years of age. In our study males and females were equal in number (50 each).[12]

Cervical region was the most frequent site of lymphadenopathy in our study (67%). Studies by S.S.Ahmed et al [73.5%] in 2005,[13] Sumyra K Q et al [76%] in 2012[14] also found cervical region to be the most common site of involvement. EPTB not only involved cervical group of lymph nodes, but also axillary, inguinal nodes. This is in agreement with study by A Hemalatha et al in 2011 where they found similar findings.[15]

In the present study, majority (98%) of the cases clinically suspected to have EPTB were non-neoplastic and 2% were neoplastic in nature. Our findings are similar to findings of study by Arjun singh et al in 2013 where 85.4% were non-neoplastic and 14.6% were neoplastic.[16]

Among 47 cases with FNAC findings suggestive of tubercular etiology, epithelioid granuloma without necrosis accounted for 22%, epithelioid granuloma with necrosis accounted for 13% and caseous necrosis without epithelioid cells accounted for 12%. Among these 47 cases CB-NAAT was positive in 36.6% of cases showing epithelioid granuloma with caseous necrosis, 33.3% of cases showing caseous necrosis without epithelioid cells and 30.0% of cases showing epithelioid granuloma without caseous necrosis.

CB-NAAT was positive in 5 cases (14.2%) which did not show findings favouring tubercular etiology by FNA. Those cases would have been missed if CB-NAAT was not done.

We have taken CB-NAAT as the standard for comparing the validity of FNAC and AFB positivity. So the predictive validity of FNAC compared to CB-NAAT had sensitivity of 85.7%, specificity of 73.8%, false positive rate of 26.1%, false negative rate of 14.2%, PPV of 63.8%, NPV of 90.5%, diagnostic accuracy of 78%. This is in comparison with study by Tadesse et al in 2015 where they have calculated the diagnostic accuracy of cytology compared to composite reference standard who reported sensitivity of 80%, specificity of 57.8%, PPV of 79.1%, NPV of 59.1%. To the best of our knowledge this is first study to compare

Table 5: Comparison of CB-NAAT result with FNAfindings suggestive of Tubercular etiology (n=100)

FNA diagnosis	CB-NAAT Result		Chi square	p value
	Positive (n=35)	Negative (n=65)		
Positive (47)	30 (85.7%)	17 (26.1%)	32.3	<**0.001**
Negative (53)	5 (14.2%)	48 (73.8%)		

Table 6: Predictive validity of FNAC compared to CB-NAAT (n=100)

Parameter	Value (%)	95% CI	
		Lower	Upper
Sensitivity	85.7	69.7%	95.1%
Specificity	73.8	61.4%	83.9%
False positive rate	26.1	16.0%	38.5%
False negative rate	14.2	4.8%	30.2%
PPV	63.8	48.5%	77.3%
NPV	90.5	79.3%	96.8%
Diagnostic accuracy	78.0	68.6%	85.6%

Table 7: Summary of CB-NAAT positive and AFB positive cases with FNAC features (n=13)

FNAC features	CB-NAAT and AFB positive cases (n=13)	Percentage (%)
Epithelioid granuloma with caseous necrosis	08	61.5
Epitheloid granuloma without caseous necrosis	01	7.6
Caseous necrosis without epithelioid cells	02	15.3
Suppurative lesion	02	15.3

Table 8: Comparison of CB-NAAT RESULT with AFB (n=50)

AFB	CB-NAAT Result	
	Positive (n=35)	Negative (n=65)
Positive	13 (37.1%)	0 (0%)
Negative	22 (62.8%)	65 (100%)

Table 9: Predictive validity of AFB compared to CB -NAAT (n=100)

Parameter	Value (%)	95% CI	
		Lower	Upper
Sensitivity	37.1	21.4%	55.0%
Specificity	100.0	94.4%	100.0%
False positive rate	0.0	0.0%	5.5%
False negative rate	62.8	44.9%	78.5%
Positive predictive value	100.0	75.2%	100.0%
Negative predictive value	74.7	64.2%	83.4%
Diagnostic accuracy	78.0	68.6%	85.6%

FNAC, AFB positivity and CB-NAAT findings.

Among 35 cases which were CB-NAAT positive, 13 (37.1%) were AFB positive in the FNAC smears and majority of them showed epithelioid granuloma with caseous necrosis 61.5%, while 15.3% each showed caseous necrosis without epithelioid cells and suppurative lesion, 7.6% showed epithelioid granuloma without necrosis.

The predictive validity of AFB in CB-NAAT result is 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%, diagnostic accuracy of 78%.

To conclude, 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.

6. Acknowledgement

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7. Conflict of Interest

None.

8. Source of Support

There is no financial assistance from any source for this study.

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