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Original research article

Original research article: Evaluation of Extra Pulmonary Tuberculosis (EPTB) cases with CBNAAT in comparison with cytology

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Abstract

Introduction: EPTB contributes to a significant burden of mortality and morbidity, due to its complex and sub clinical presentations, leading to a delay in their diagnosis. EPTB has negligible contagious potential and therefore never been prioritised in the campaigns undertaken by National TB Control Programs. Various methods can be used for the detection of EPTB of which microscopy, culture and identification of the organism's DNA are known. Newer method known is CBNAAT. Present study was proposed to evaluate the effectiveness of CBNAAT in diagnosing EPTB in comparison with cytology.
Materials and Methods: The study included 120 samples of lymph node, pus, pleural fluid, ascitic fluid and C.S.F. Samples were collected and subjected to AFB smear, FNAC and CBNAAT simultaneously.
Results: Among total 120 cases, EPTB diagnosed by CBNAAT, FNAC, AFB stain were 40, 71 and 18 respectively.
Conclusion: CBNAAT is a newer confirmatory test and adds an extra edge of simultaneous drug resistance. Still combining CBNAAT with other tests provide better diagnostic yield even at peripheral places.
Keywords: CBNAAT, extra pulmonary tuberculosis, FNAC, AFB stain

Introduction

Tuberculosis remains a worldwide public health problem, despite the fact that the causative organism was discovered more than 100 years ago and there are many highly effective drugs and vaccine available, making tuberculosis a preventable and curable disease. The control of tuberculosis was started with advent of BCG, new drugs, improvements in the standard of living and the quality of life of the people and the utility of health resources. Present study was proposed to assess the effectiveness of CBNAAT in diagnosing EPTB in comparison with that of cytological examination. It is aimed to define the role of CBNAAT in clinical decision-making in suspected EPTB cases [1], weather CBNAAT is helpful in clinically suspected cases of tuberculosis where acid fast bacilli (AFB) staining is negative [2]. CBNAAT is a rapid and fully automated NAAT (Nucleic Acid Amplification Test), also known as Xpert MTB/RIF assay. CBNAAT was adopted in India by RNTCP in 2012. The Xpert MTB/RIF assay is based on hemi-nested real-time PCR amplifying the rpoB gene target. It is recommended that Xpert MTB/RIF is used for the diagnosis of EPTB, for suspected cases of pulmonary TB (conditional recommendations) and TB in children.

Materials and methods

Current Study is a Prospective cross-sectional study done at Department of pathology, Rangaraya Medical College, Kakinada for a period of 18 months from March 2021 to August 2022 on 120 cases.
Inclusion criteria: All clinically suspected cases of Extra Pulmonary Tuberculosis.
Exclusion criteria: Cases of exclusive Pulmonary Tuberculosis and Cases with inadequate aspirate.

After taking detailed history, examination findings and available investigation, results were documented in a pre-designed proforma. For lymph node and cold abscess FNA was performed. Four smears were made, two for Haematoxylin and Eosin(H&E) stain, one for May Grunwald Giemsa (MGG) and one air

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dried smear for AFB stain. Needle washes were collected in 0.9% Normal saline for CBNAAT simultaneously. FNA was repeated if aspirate was found to be inadequate. The aseptically collected aspirate was placed in a sterile container with normal saline to avoid drying of the material and labelled was immediately sent for CBNAAT. For fluid samples they are centrifuged and smeared similarly.

Statistical analysis

Results were tabulated and analysed using SPSS software 23 version.

Results

Among 120 cases of EPTB, most of the patients (25%) were of 21-30 years age. Men seem to be affected more and constituted 59.2% of the patient group. Most of the cases were from lymph node (59.2%) and pleural fluid cases (26.7%). Cytology was suggestive of tuberculosis in 71 cases, AFB stain in 28 cases, CBNAAT in 40 cases. CBNAAT tested marginally more positive than AFB stain. Out of 28 positive HIV cases 14,10 and 5 cases were positive for tuberculosis on cytology, AFB and CBNAAT respectively.

Table 1: Distribution of HIV status among CBNAAT

	CBNAAT Positive		CBNAAT Negative		Total
	Cases	Percentage	Cases	Percentage	
HIV Positive	12	42.9%	16	57.1%	28
HIV Negative	28	30.4%	64	69.6%	92
Total	40		80		120

Table 2: Comparison table of CBNAAT with AFB stain

AFB staining	CBNAAT		Total
	Positive	Negative	
Positive	18	6	24
Negative	22	74	96
Total	40	80	120

On comparison of CBNAAT and AFB staining (table 2),18 cases were detected positive in both the tests, whereas 22 subjects who were detected positive on CBNAAT were negative for AFB, 6 case who were negative on CBNAAT was positive for AFB. The sensitivity, specificity, PPV and NPV of CBNAAT was found to be 45%, 92.50%, 75% and 77.08% respectively.

On comparison of CBNAAT and cytology, 24 cases were detected positive in both tests, whereas 16 cases were positive on CBNAAT and negative on cytology. 47 cases which were negative in CBNAAT were positive on cytology. The sensitivity, specificity, PPV and NPV of CBNAAT was found to be 60%, 41.25%, 33.8% and 67.35% respectively.

Table 3: Result of CBNAAT, FNAC, AFB stain among various samples

EPTB cases	Total samples	FNAC positive	CBNAAT positive	AFB positive
Lymph node	71	51	26	16
Pleural fluid	32	12	6	2
Pus	11	7	6	6
Ascitic fluid	4	1	0	0
Cerebrospinal fluid	2	0	2	0
Total	120	71	40	24

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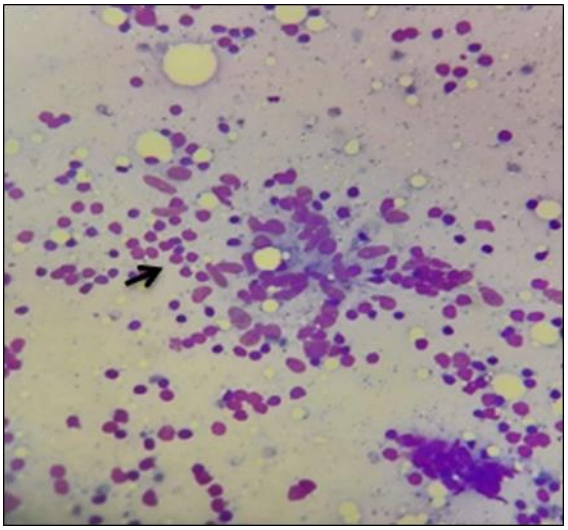

Image 1: 400x MGG stain epithelioid cell cluster

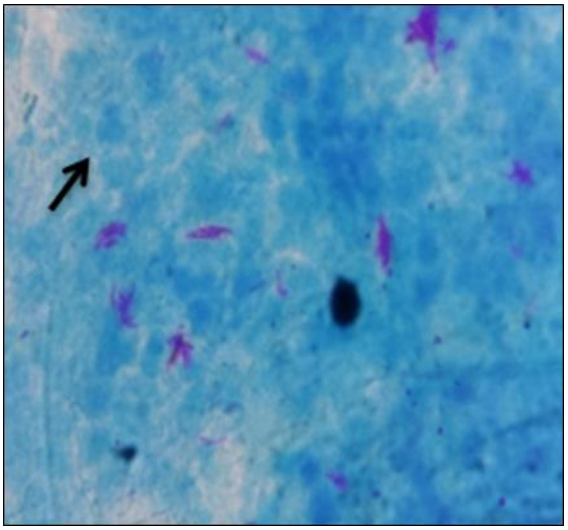

Image 2: 1000x AFB stained bacilli

Discussions

In the present study, the patients who were suspected to have EPTB belongs to a range of age between 6months-80 years with predominant age group of 21-30 years, constitute of 25%. The results were comparable with the studies done by Komanapalli SK *et al.* [3] where they had maximum cases with a wide range of age group of 11-30 year, K Arpitha *et al.* [4] study 29.4% cases. In Dronadula *et al.* [5] study there is 39% of cases are seen in the age group of 20-39 years.

Male predominance of 59.2% was seen in current study which are comparable with Mukherjee *et al.* [6], Singh KG *et al.* [7], K Arpitha *et al.* [4], Thakkar B *et al.* [8] and Rameshbabu B *et al.* [9] studies. This indicates more awareness among males when compared with females or more number of male patients were affected in the study group.

Among various samples maximum percentage of cases 59.2% were from lymph node mostly from anterior cervical group. 26.7% were pleural fluids. A study done by Tortoli *et al.* [10], had 24% pleural effusion cases, Causse *et al.* [11] had 19.4% of cases of pleural or other body fluids. When compared with these studies done on EPTB samples percentage of pleural fluid cases are more. 3.3% of samples were of ascitic fluid, CSF is of 1.7% cases. Pus samples (cold abscess samples, swellings, subcutaneous abscess and few with multiple discharging sinuses) 9.2% of the cases.

Study by Kandi *et al.* [12] pulmonary samples were 55%, extrapulmonary were 45%, out of them lymph node samples were 19%, pleural fluid and BAL fluid samples were 10%, CSF samples were 2.5%. In Dronadula *et al.* [5] study most of the samples are taken from lymph node aspirate which constitute major portion among all samples and among EPTB samples.

In our study among lymph nodal and pus sample cases CBNAAT and FNAC positive cases are (21/82) 25.60%. 11 cases out of 82 cases (13.41%) are FNAC negative which are benefited by turning to be positive in CBNAAT. But in (14/82) 17.07% of cases are detected in FNAC where CBNAAT have failed to detect the bacilli as this may be due to last passage sample sent for CBNAAT which may yield less amount of sample when sent for CBNAAT in normal saline it may be excessively diluted [13]. Normally extra pulmonary samples are mostly pauci bacillary if there is very less aspirate this may lead to CBNAAT negative sample even though the FNAC is positive.

In present study both CBNAAT and FNAC negative cases (36/82) constitute 43.90% which is very high, lead to more specificity of the tests. Among all lymph node and pus samples which accounting for 82 cases the CBNAAT and FNAC showed sensitivity of 65.62% and specificity of 72%, which is comparable to sensitivity of Aruna L *et al.* [14] study.

In the present study, 18 cases were detected positive in AFB and CBNAAT, whereas 6 were positive on AFB (shown in image 2) and negative on CBNAAT. This may be due to thick caseous material which yielded less aspirate on FNAC, leading to excess dilution of sample for CBNAAT leading to negativity of sample. 22 cases positive on CBNAAT were negative for AFB. This indicates the benefit of CBNAAT in the paucibacillary samples. On calculating chi-square statistics among CBNAAT and AFB the value is 23.4375 and the p-value is <0.00001.

In a study done by Mukherjee *et al.* [6] Lymph node FNA showed AFB smear positivity in 28 out of 114 cases (24.6%) and five out of eleven (45.5%) cases of cold abscess aspirate. In Manju Kumari *et al.* [15] study 41% CBNAAT positive and 24% by AFB staining positive. 17% cases were detected with tuberculosis with the help of CBNAAT only and were reported as negative on AFB stain. In a study conducted by Gupta H *et al.* [60] AFB positivity was 14/50 cases (28%) while CBNAAT positivity was 32/50 cases (64%). Patil SB *et al.* [17] study showed, 46.35% cases were positive on AFB stain while 55.20% cases were positive for CBNAAT among 192 diagnosed cases of EPTB cases.

In the present study, 18 cases positive in AFB and Cytology, 6 cases were positive in AFB turned

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negative in cytology. 53 cases negative for AFB were positive on cytology. The sensitivity, specificity, PPV and NPV of AFB with cytology was found to be 25.35%, 87.76 %, 75% and 44.79% respectively when compared to cytology. In a study done by Gundrajakuppam *et al.* [18], the sensitivity, specificity, PPV and NPV of AFB stain with FNAC was found to be 12.5%, 97.1%, 83.3% and 49.6% respectively. In a study done by, Vishnu *et al.* [19], Tamanna *et al.* [20] the sensitivity & specificity of AFB stain against composite diagnostic methods was 23.08% & 100% respectively.

AFB positivity is low in epthelioid granulomas without necrosis (5.8%-30.0%), but significantly higher in epithelioid granuloma with necrosis (32.0%-64.7%). It is highest in necrosis without epithelioid granulomas (48.5%-77.4%) [21]. This indicates that CBNAAT among abscess cases show maximum positivity, with maximum bacillary load as mostly the abscess contain caseous necrosis [21].

All the studies showed high specificity for detection of AFB on ZN stain which is similar to our present study. The quality of the smear as well as the paucibacillary nature of the fine needle aspirate could be the main factor for decreased sensitivity, but as expected, the specificity was the highest.

Out of total 28 HIV positive cases (table 2), 42.9% CBNAAT positive cases were detected. The sensitivity is about 30% in the people living with HIV infection. CBNAAT is negative in 57.1% of HIV cases. In Komanapalli SK *et al.* [3] study among EPTB with HIV cases the sensitivity is 78.95%, specificity is of 50%. The sensitivity of CBNAAT among HIV cases is high when compared with current study. In the present study total number of HIV cases is high as there is high level of suspicion among them clinically.

There are 9 CBNAAT negative cases among HIV patients who turned positive on FNAC which have to be further confirmed by using culture. Out of 12 CBNAAT positive cases, 7 cases are negative in FNAC. Komanapalli SK *et al.* [3] study showed very less HIV positive cases. Kandi S *et al.* [12] study show 6% of CBNAAT positive cases with HIV.

The CBNAAT positivity among lymph node is 36.6%, 18.7% of pleural fluid, 54.5% of pus,100% of CSF cases in current study from table 1. Rameshbabu B *et al.* [9] study showed CBNAAT positivity of lymph nodal, other swellings 29.7%, pleural fluid 10.9%, pus & abscesses 26.82%, ascitic fluid 0.3%, CSF 0.02%, others 28.7%. Dr Prayas *et al.* [21] study showed CBNAAT positivity in 66.2% cases, AFB positivity in 47.5% cases and FNAC positivity in 72.5% cases among 80 cases of EPTB samples.

Diagnosing EPTB is challenging due to its varied clinical presentations and paucibacillary nature of the disease [2]. AFB smear hasn't been proved to be much useful in diagnosing EPTB. CBNAAT is nucleic acid amplification test which detects the TB bacilli and simultaneous resistance to Rifampicin. CBNAAT is likely to revolutionize the diagnosis and treatment of EPTB, as it is a very cost-effective and rapid test.

Conclusion

CBNAAT is a conformatory test and very helpful test in detecting EPTB cases as it can detect bacilli when present in less number and also drug resistance. CBNAAT is more effective method than AFB stain in identification of EPTB among fluids. In early suspicious cases of lymph node the best method is FNAC. Inclusion of CBNAAT in initial diagnosis of EPTB cases will reduce the injudicious use of anti-tuberculosis drugs. Thus, when CBNAAT is combined with cytological examination simultaneously, it will be helpful in accurate diagnosis and early treatment of EPTB.

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List of Abbreviations

AFB	Acid Fast Bacilli
BCG	Bacillus Calmette-Guerin
CBNAAT	Cartridge Based Nucleic Acid Amplification Test
CSF	Cerebro spinal fluid
EPTB	Extra pulmonary tuberculosis
FNAC	Fine Needle Aspiration Cytology
H & E	Haematoxylin and Eosin
HIV	Human Immunodeficiency Virus
MGG	May Grunwald Giemsa
NAAT	Nucleic Acid Amplification Test
NPV	Negative Predictive Value
PCR	Polymerase Chain Reaction
PPV	Positive Predictive Value
TB	Tuberculosis
WHO	World Health Organization
Xpert MTB/RIF	Cartridge based nucleic acid amplification test for diagnosis of Mycobacterium tuberculosis and rifampicin resistance.
ZN stain	Zeihl-Neilson stain