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Diagnostic Yield of Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) In Lymph Node Tuberculosis at Institute of Respiratory Disease, SMS Medical College, Jaipur

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Abstract:
Introduction: *Lymph Node Tuberculosis (LNTB) is considered to be the local manifestation of a systemic disease. LNTB often affects children and young adults. Mycobacterial lymphadenitis most frequently affects patients in their second decade but may affect patients of any age. Some patients with LNTB may manifest systemic symptoms and these include fever, weight loss, fatigue, and night sweats. There are many methods for detecting LNTB in patients, but recently focus has been shifted to Molecular diagnosis or nucleic acid amplification (NAA). The purpose of the study was to evaluate the diagnostic yield of CBNAAT in LNTB.*
Materials And Methods: *This study was conducted at Institute of Respiratory diseases, SMS Medical college, Jaipur, Rajasthan, India from June 2017, to June 2018. In total, 51 consecutive patients, with suspicion of LNTB were subjected fine needle aspiration (FNA) and the specimens were processed simultaneously for Ziehl-Neelsen, Cytopathology, liquid culture via 'NAP test' or 'TB Ag MPT4 Rapid' immunochromatographic assay and CBNAAT.*
Results: *Among 51 samples tested, CBNAAT detected DNA of MTBC in 37 samples (72.5%). Highest yield was found with cytopathology, with detecting caseous necrosis and AFB in 38 samples (86.4%). Standard biological assays, including AFB microscopy and culture, were positive, respectively, in 20 (39.2%) and 25 (49%). FNA CBNAAT sensitivity and specificity were assessed according to smear and culture results, clinical and cytopathological findings. The sensitivity and specificity of CBNAAT were 77.27% and 57.1% respectively.*
Conclusion: *Combining FNAC and rapid genotypic diagnosis using automated systems (CBNAAT) should greatly improve access to appropriate diagnosis and treatment for patients with tuberculous lymphadenitis*.
Keywords: *CBNAAT, FNAC Of lymph Node, Mycobacterial lymphadenitis,*

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I. Introduction

Tuberculosis can involve any organ system in the body.[1] While pulmonary tuberculosis is the most common presentation, extrapulmonary tuberculosis (EPTB) is also an important clinical problem.[1] The term EPTB has been used to describe isolated occurrence of tuberculosis at body sites other than the lung.[1] In India and other developing countries, LNTB continues to be the most common form of EPTB and lymphadenitis due to non-tuberculous mycobacteria (NTM) is seldom seen.[1] On the other hand, NTM is the most common cause of lymphadenopathy in the developed world.[1] TB lymphadenitis is seen in nearly up to 40% of extrapulmonary TB which constitutes approximately 15–20% of all cases of TB in India.[2] Conventional MTB detection techniques, based on microscopic examination of Ziehl–Neelsen or auramine-stained specimens and culture, are required for diagnostic confirmation, but they fail to provide an optimal sensitivity. So, in recent times, attention has been devoted to new nucleic acid amplification diagnostic technologies, owing to their rapidity, sensitivity, and specificity.[3]

The purpose of the study was rapid diagnosis of mycobacterium tuberculosis and rifampicin resistance in clinically suspected cases of lymph node tuberculosis and comparing diagnostic yield of CBNAAT with conventional methods.

II. Materials And Methods

Study design and Specimens

This was tertiary hospital based, descriptive type of observational study conducted at IRD, SMS Medical college, Jaipur, Rajasthan, India from June 2017 to June 2018. This study was approved by the Ethics committee and Institutional Review Board, SMS Medical College, Jaipur.

Suspected cases of LNTB and giving informed consent were included.

Lymph node enlargement with strong suspicion of other disease and active pulmonary cases were excluded.

Lymph node specimens (tissues and aspirated) were consecutively collected from 51 patients with clinically suspected case of LNTB at IRD, SMS Medical college, Jaipur.

Three specimens by FNA were collected; the first was sent for ZN staining. Second was sent for CBNAAT and MTB culture. The third one was sent for cytopathology.

Mycobacterial smear

Air fixed slides were used for AFB smear at our institute. ZN staining was used to analyse AFB smear. The procedure of staining was according to guidelines issued by RNTCP. Reporting and grading was also done according to RNTCP guidelines.

Mycobacterial culture

It was carried out at department of microbiology, in our institute using 'BACT/ALERT 3D' machine. 2ml of FNA sample was sent in sterile container. Positive or negative culture report was issued after 45 days. It was done using BACT/ALERT 3D machine at department of microbiology in our institute. All positive culture samples were subjected to either 'NAP test' or 'TB Ag MPT4 Rapid' immunochromatographic assay to differentiate between Mycobacterium tuberculosis complex (MTBC) and Mycobacteria Other Than Tuberculosis (MOTT).

Cytopathology

It was carried out at department of pathology in our institute.

CBNAAT

For the CBNAAT, a 2ml FNA sample was poured into a single use disposable cartridge that is placed in to Xpert Dx module with the results produced is less than 2 hours. Each PCR run comprised an internal control for sample processing (DNA extraction) and PCR validity (presence of inhibitors), with positive and negative controls tested every day. The system automatically interpreted all results from measured florescent signals, with embedded calculation algorithms, into the following categories: invalid, if PCR inhibitors are detected with amplification failure; negative or positive. If positive, the strain was categorized as susceptible or resistant to rifampicin.

Patient categories

Patients were categorized into 4 groups: "confirmed TB" cases (culture positive, smear negative or positive),

"probable TB"cases (culture negative, but showing clinical symptoms, smear positive and histology/cytology suggestive of TB), "possible TB" cases (negative culture, but showing clinical symptoms, and negative smear and histology/cytopathology suggestive of TB), and "not TB" cases (only clinical symptoms).

III. Results

This study included 51 patients. The male to female ratio was 0.65 (20/31). The median age of the patients for TB was 32.07yrs and for non-TB 24yrs. All patients were found to be HIV negative. Pain was complained by only 3 patients. Left cervical LN was most common presentation followed by right cervical LN. Constitutional symptoms; loss of weight 33 (75%) and loss of appetite 30 (68.2%) were most commonly found in tubercular as

well as non-tubercular group. Most of the patients didn't have any contact history with tubercular patient.

FNA AFB smears were positive for 20(45.5%) patients with TB. Among non-TB patients, AFB was negative in all 7 patients; giving sensitivity and specificity of 45.45% and 100% respectively (table 1).

TABLE 1

FNA AFB finding	Final diagnosis				Total	
	Tuberculosis		Non-Tuberculosis			
	N	%	N	%	N	%
Positive	20	45.5	0	0	20	39.2
Negative	24	54.5	7	100	31	60.8

Total	44	100	7	100	51	100

FNA CBNAAT was found positive in 34 (77.3%) of patients and negative in 10 (22.7%) in LNTB; giving a high sensitivity (77.27%) but low specificity (57.1%). Negative predictive value was 28.57%, This indicates that TB cannot be ruled out on the basis of CBNAAT. Overall diagnostic accuracy was 74.51%. Hence, CBNAAT
could be used as one of the first tools to detect TB in these patients, but if CBNAAT is negative other tests should be carried out for confirmation (table 2).

TABLE 2

CBNAAT Finding	Final diagnosis				Total	
	Tuberculosis		Non-Tuberculosis			
	N	%	N	%	N	%
Positive	34	77.3	3	42.9	37	72.5
Negative	10	22.7	4	57.1	14	27.5
Total	44	100	7	100	51	100

In the study, it was found that sensitivity of CBNAAT (77.27%) was significantly higher (p=0004) as compared to FNA AFB (45.45%). The overall diagnostic accuracy of CBNAAT was also significantly higher as compared to FNA AFB (p=0.039) (table 3).

TABLE 3

Variable	FNA - AFB	FNA - CBNAAT	P value
Sensitivity	45.45% (30.39 – 61.15%)	77.27% (62.16 – 88.53%)	0.004 (S)
Specificity	100% (59.04 – 100%)	57.1% (18.41 – 90.10%)	0.558 (NS)
PPV	100%	91.89% (82.60 – 96.44%)	<0.001 (S)
NPV	22.58% (18.21 – 27.64%)	28.57% (14.70 – 48.14%)	0.954 (NS)
Accuracy	52.94% (38.46 – 67.07%)	74.51% (60.37 – 85.67%)	0.039 (S)

Culture was positive in 25 (56.8%) with TB. In non-TB patients, culture was negative in all 7 patients. On comparison with CBNAAT, later had a higher sensitivity and overall diagnostic accuracy was also higher (table 4)

TABLE 4

Variable	Culture	FNA - CBNAAT	P value
Sensitivity	56.82% (41.03 – 71.65%)	77.27% (62.16 – 88.53%)	0.070
Specificity	100% (59.04 – 100%)	57.1% (18.41 – 90.10%)	0.192
PPV	100%	91.89% (82.60 – 96.44%)	0.392
NPV	26.92% (20.79 – 34.08%)	28.57% (14.70 – 48.14%)	0.497
Accuracy	62.75% (48.08 – 75.87%)	74.51% (60.37 – 85.67%)	0.332

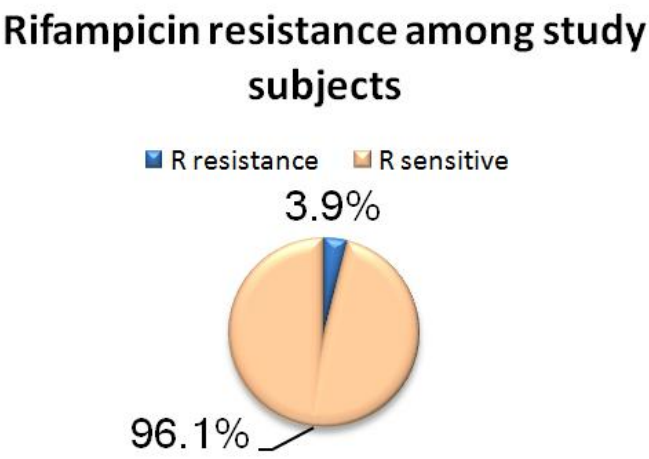
Cytopathology was positive in 38 (86.4%) in TB patients and negative in all 7 non-TB patients. On comparing with CBNAAT, cytopathology had a higher sensitivity, but the difference was not statistically significant. And overall diagnostic accuracy was also higher with cytopathology (table 5).

TABLE 5

Variable	Cytopathology	FNA - CBNAAT	P value
Sensitivity	86.36% (72.65 – 94.83%)	77.27% (62.16 – 88.53%)	0.122
Specificity	100% (59.04 – 100%)	57.1% (18.41 – 90.10%)	0.192
PPV	100%	91.89% (82.60 – 96.44%)	0.392
NPV	53.58% (35.68 – 71.05%)	28.57% (14.70 – 48.14%)	0.224
Accuracy	88.24% (76.13 – 95.56%)	74.51% (60.37 – 85.67%)	0.344

Also, rifampicin resistance was found in 2 (3.9%) patients with CBNAAT (figure 1).

FIGURE 1



IV. Discussion

Worldwide, Tuberculosis is one of the top 10 causes of death and the leading cause from a single infectious agent.[4] There are nearly 9 million new cases and 2 million deaths from tuberculosis worldwide every year in 2017, TB caused an estimated 1.3 million deaths among HIV-negative people and there were an additional 300000 deaths from TB among HIV-positive people.[4] About 1.7 billion people, 23% of the world's population, are estimated to have a latent TB infection, and are thus at risk of developing active TB disease during their lifetime.[4]

Tuberculosis can involve any organ system in the body.[1] While pulmonary tuberculosis is the most common presentation, extrapulmonary tuberculosis (EPTB) is also an important clinical problem.[1] The term EPTB has been used to describe isolated occurrence of tuberculosis at body sites other than the lung.[1] However, when an extrapulmonary focus is evident in a patient with pulmonary tuberculosis, such patients have been categorized under pulmonary tuberculosis as per the guidelines of the World Health Organization (WHO).[1]

Conventional MTB detection techniques, such as AAFB microscopy (Ziehl–Neelsen or auramine-stain) and culture of MTB (on solid and liquid media) are required for diagnostic confirmation of lymph node tuberculosis, but they fail to provide an optimal sensitivity.[3]So, in recent times, attention has been devoted to new nucleic acid amplification diagnostic technologies (CBNAAT), owing to their rapidity, sensitivity, and specificity.[3]

The present study is an observational and cross-sectional study. Total fifty-one patients with suspected lymph node TB were included in this study. Majority of the study population comprised of female gender with the male: female ratio of 1:1.5. Most (40.9%) of the patients having TB were from the age group of 21-30 years. The mean age of patients in the present study at the time of presentation was more for TB (32.07 ± 11.86 years) while mean total duration of illness on presentation was 4.7 ± 2.5 months. Left cervical was the most common site of involvement followed by right cervical. Most of the patients had only one site of involvement. Among all presenting constitutional symptoms loss of weight 33 (75%) and loss of appetite 30 (68.2) were the common.

Nearly all patients in both tubercular (90.9%) and non-tubercular group (85.7%) had no past history of contact with TB patient. Similarly, most of the patient in both tubercular (90.9%) and non-tubercular group (100%) had no history of ATT intake. Mean weight of TB patients was 39.09 ± 7.56 Kg. Firm consistency was the most common clinical finding (66.7%) in both TB and non-TB patients, followed by matting (56.9%). Tenderness and sinus formation were rare. Most patients in both the group had no chest X-ray findings.

FNA was performed in all 51 patients. Results of which revealed confirmed TB in nearly half of the patients (45%), 14 (27.5%) had possible TB, 5 (9.8%) had probable TB and only 7 (13.7%) were Non-TB patients. 25 patients showed positive mycobacterial cultures out of the 51 patients. With a mycobacterial culture as a gold standard, FNA AFB smear had a sensitivity of 45.45% (20/51), FNAC had a sensitivity 86.36% (38/51) and FNA CBNAAT showed a sensitivity of 77.27% (37/51).

CBNAAT OF FNA samples of lymph node was superior in terms of sensitivity (77.27% vs 45.45%) and accuracy (74.51% vs 52.94%) when compared to FNA smear microscopy' when compared to FNA MTB culture CBNAAT of FNA samples of lymph node was superior in terms of sensitivity (77.27% vs 56.82%) and accuracy (74.51% vs 62.75%).

CBNAAT OF FNA samples of lymph node were almost similar in terms of sensitivity (77.27% vs 86.36%) and accuracy (74.51% vs 88.24%) when compared to FNAC. Additionally, CBNAAT also detected Rifampicin resistance in 2 (3.9%) patients, making it a good diagnostic tool for early detection of MDR cases

V. Conclusion

Tuberculosis is a systemic disease and lymphadenitis is the most common extra pulmonary manifestation of the disease. Diagnosis need a high index of clinical suspicion and application of a variety of diagnostic modalities. FNA is a simple procedure which can be performed in an outpatient setting by clinicians. Combining FNAC and rapid genotypic diagnosis using automated systems (CBNAAT) should greatly improve access to appropriate diagnosis and treatment for patients with tuberculous lymphadenitis.

Implementation of CBNAAT test in TB endemic settings could significantly improve the rapid diagnosis of lymph node tuberculosis along with early recognition of rifampicin resistant cases.

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