European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 09, Issue 08, 2022

Original Research Article

Study Of Diagnostic Accuracy Of CBNAAT In Bronchial Washings Of Sputum Smear-Negative And Sputum-Scarce Patients With Presumptive TB

[1]Dr. Sukumar KN, [2]Dr. Nandish C, [3]Dr. Spurthi K, [4]Dr. Hasna Erattengal

[1,3,4]Senior Resident, Department of Pulmonary Medicine, JJM Medical College, Davangere, Karnataka, India

[2]Assistant Professor, Department of Pulmonary Medicine, JJM Medical College, Davangere, Karnataka, India

Corresponding Author:
Dr. Nandish C

ABSTRACT

A rapid, accurate, and reliable diagnostic test is needed for these patients so that early anti-tubercular treatment (ATT) can be started for preventing the transmission of TB and the clinical severity of the disease. Moreover, unnecessary treatment can be avoided in patients who do not have TB with the avoidance of additional expenditure on medication. Patients who are clinically suspected to have pulmonary tuberculosis, including those who have symptoms of cough with or without expectoration for >2 weeks, weight loss, fatigue, hemoptysis, and loss of appetite with sputum smear-negative for PTB were included for the study. Among the 80 cases studied, the total number of PTB cases diagnosed was 58 (72%). The total number of PTB cases being CBNAAT +ve was 52. The total number of PTB cases having positive LJ culture was 58 cases. The total number of cases, of other diseases diagnosed, was 22, which included 2 cases of malignancy (1-squamous cell carcinoma, 1-adenocarcinoma), 4 cases of fungal (mucormycosis and aspergilloma) pneumonia, and 16 cases of bacterial (Klebsiella, pseudomonas, CONS) pneumonia. The sensitivity of CBNAAT was 87.93%, specificity was 95.45%, positive predictive value 98.07%, Negative predictive value-75%.

Keywords: CBNAAT, bronchial washings, presumptive TB

INTRODUCTION

Diagnosing extrapulmonary tuberculosis (EPTB) remains a considerable challenge as the bacilli remain deep-seated within the tissue site of the disease and often very low viable clinical specimens are obtained**.** The use of Histology in diagnosing TB with high specificity is a time-consuming task and not a feasible method. Tissue microscopy after special staining is often negative and when mycobacteria are seen, it is impossible to distinguish MTB from

ISSN 2515-8260 Volume 09, Issue 08, 2022

non-tubercular mycobacterial disease, and Reliance on culture, the mainstay of diagnosis, often leads to considerable delays, hindering the patient care and outcomes [1].

A rapid, accurate, and reliable diagnostic test is needed for these patients so that early anti-tubercular treatment (ATT) can be started for preventing the transmission of TB and the clinical severity of the disease. Moreover, unnecessary treatment can be avoided in patients who do not have TB with the avoidance of additional expenditure on medication [2].

To address this issue The X-pert MTB/RIF a Cartridge based nucleic acid amplification test (CBNAAT) assay was rapidly endorsed by the WHO in December 2010, for early diagnosis of tuberculosis in less than two hours, with special emphasis on drug-resistant tuberculosis, human immunodeficiency virus (HIV), and TB co-infection, pediatric tuberculosis, extrapulmonary tuberculosis, and smear-negative pulmonary tuberculosis. "The advent of gene X-pert has resulted in a paradigm shift in the diagnosis of TB". "CBNAAT-cartridge-based nucleic acid amplification test the rapid, fully automated NAAT also known as X-pert MTB/RIF assay-has been described as a breakthrough in the TB control program. Gene X-pert is currently the only one of its kind using a cartridge containing lyophilized reagents, buffers and washes. The X-pert MTB/RIF assay is based on hemi-nested real-time PCR amplifying the rpoB gene target". In the 2013 updated policy, WHO recommended the use of X-pert MTB/RIF for the diagnosis of EPTB, for suspected cases of pulmonary TB (conditional recommendations) and TB in children [3].

CBNAAT Testing involves three manual steps:

1. The sample is treated with a reagent to inactivate and liquefy the bacteria in sputum.
2. Two ml of this liquefied sputum is added to cartridge.
3. The cartridge is then loaded into the test device for the assay.

The following steps are fully automated. The role of CBNAAT for the early diagnosis of tubercular effusion has been evaluated as an alternative diagnostic tool with the added advantage of detecting rifampicin resistance [4].

This study was conducted to better the understanding of diagnostic accuracy of CBNAAT in bronchial washings of sputum smear-negative and sputum-scarce patients with presumptive TB.

Methodology

A patient of tuberculosis-suspect, based on clinical and radiological features, compatible with a diagnosis of pulmonary tuberculosis. A smear-negative case was one in which two consecutive early morning sputum samples did not reveal acid-fast bacilli when examined by microscopy with Zeihl-Nelson stain. Patients who had less than 1 ml of sputum were defined as having poor sputum-scarce disease. A confirmed case of pulmonary tuberculosis was one in which Mycobacterium tuberculosis (MTB) grew on mycobacterial cultures by solid or liquid culture medium, which was taken as the gold standard. Patients of either gender aged above 12 years of age, that had suspected pulmonary tuberculosis on clinical or radiological grounds, were included in the study. Smear-positive cases, those with disseminated or extrapulmonary tuberculosis patients were excluded from the study. Following written consent for bronchoscopy, demographic and clinical data were collected. Bronchoscopy was performed by trans nasal route and bronchoscope was wedged into the sub-segmental bronchus of interest and Bronchial wash was obtained by instillation of sterile normal saline.

ISSN 2515-8260 Volume 09, Issue 08, 2022

It was sent for ZN stain and mycobacterial liquid culture (gold standard), for CBNAAT to detect Mycobacterium tuberculosis (MTB) and rifampicin resistance.

Inclusion criteria

1. Patients who are clinically suspected to have pulmonary tuberculosis, including those who have symptoms of cough with or without expectoration for >2 weeks, weight loss, fatigue, hemoptysis, and loss of appetite with sputum smear-negative for PTB.
2. Patients with radiological features suggestive of pulmonary tuberculosis with smear-negative for PTB.
3. Patients unable to expectorate mucoid sputum after induction with 3% NaCl solution.
4. Patients who are willing to get involved in the study.

Exclusion criteria

1. Microbiologically positive TB patients.
2. Patients who are unfit for FOB (severe asthma, recent MI bleeding disorders).
3. Isolated extra-pulmonary tuberculosis.
4. RVD positive patients.

Study design

Prospective cross-sectional Descriptive study.

Sample size: 80

Laboratory methods

- Following written consent for bronchoscopy, after overnight fasting, the necessary medication is given with injection TT and the test does of xylocaine, patient was nebulized before the procedure with C+D. Patients were also nebulized with 4% Xylocaine before the bronchoscopy. The bronchoscopy procedure was done transnasally with the patient lying in a supine position with constant vitals like pulse oximetry and electrocardiographic monitoring. The tip of the bronchoscope was passed till vocal cords, of 2% Xylocaine was instilled to anesthetize the vocal cords and the scope was advanced below the vocal cords into the trachea till the level of the carina, here 2 ml of 2% Xylocaine was again instilled. A thorough examination of the nasopharynx, vocal cords, and the tracheobronchial tree were done. Bronchial washings of the affected lobe where there is lesion are done with about 10-15 ml of 0.9% sterile saline (instilled with a syringe) and by application of 50-80 mm Hg negative pressure from a suction device and the fluid was collected in sterile disposable 75 ml sample traps. In the patients who did not have localized disease, on chest roentgenogram, bronchial washings were taken from the right middle lobe and the lingula. The scope was later removed carefully and the patient was advised to sleep on the affected side and given post bronchoscopy instructions.
- Each bronchial wash sample received in the lab from the centers as per the collection and

ISSN 2515-8260 Volume 09, Issue 08, 2022

transportation policy of the laboratory, were divided into two parts.

- One part was immediately tested using CBNAAT, the second part was used for MGIT BACTEC 320 liquid culture and performed on the same day.
- For Liquid culture as much as the sample was taken after sending for CBNAAT, it should be checked that the volume remaining should not be less than 2 ml for processing.
- CBNAAT testing was performed according to the manufacturer's instructions. Sample reagent was added to bronchial wash fluid at a ratio of 2:1, its shaken manually and made to rest for 10 minutes at room temperature, then shaken again and kept for 5 min; 2ml of the inactivated material was transferred to the test cartridge and inserted into the test
- The second part was processed using the N-acetyl-L cysteine- sodium hydroxide method (NALC-NaOH) as per manufacturer's instructions, cultured on MGIT medium, and incubated in the MGIT BACTEC 320 liquid culture system. Sodium hydroxide (NaOH) is a decontaminating agent and also acts as an emulsifier and NALC acts as a mucolytic agent and also reduces the concentration of NaOH required. When the tubes were flagged positive by the system, ZN staining and culture on 5% sheep blood agar were performed from the tube directly to see any contamination as per the manufacturer's instructions. All tubes were checked for positivity till 42 days. Positive tubes are stained for AFB, preferably using a carbolfuchsin method. Negative tubes are returned to the incubation rack and again observed at regular intervals for up to 6 weeks.

Results

Table 1: Diagnosis

Sl. No.	Diagnosis	Number of cases	Percentage
I	Total presumptive TB cases	80	100
A	PTB +ve With CBNAAT + MGIT	58	72
II	Total cases of other diseases diagnosed	22	27.5
A	Malignancy	2	2.5
B	Bacterial pneumonia	16	20
C	Fungal pneumonia	4	5

Among the 80 cases studied, the total number of PTB cases diagnosed was 58 (72%). The total number of PTB cases being CBNAAT +ve was 52. The total number of PTB cases having positive LJ culture was 58 cases. The total number of cases, of other diseases diagnosed, was 22, which included 2 cases of malignancy (1-squamous cell carcinoma, 1-adenocarcinoma), 4 cases of fungal (mucormycosis and aspergilloma) pneumonia, and 16 cases of bacterial (Klebsiella, pseudomonas, CONS) pneumonia.

Table 2: Distribution of study subjects as per BAL for CBNAAT

BAL for CBNAAT	Frequency	Percent
Negative	28	35.0
Positive	52	65.0
Total	80	100.0

ISSN 2515-8260 Volume 09, Issue 08, 2022

The above table shows the distribution of study subjects as per BAL for CBNAAT. 65% of subjects were positive for BAL for CBNAAT, whereas 35% of subjects were negative for BAL for CBNAAT.

Table 3: Distribution of study subjects as per Liquid culture

Liquid Culture	Frequency	Percent
Negative	22	27.5
Positive	58	72.5
Total	80	100.0

The above table shows the Distribution of study subjects as per Liquid culture. 72.5% of study subjects were positive for liquid culture whereas the rest 27.5% of subjects were negative for liquid culture.

Table 4: Correlations of liquid culture with BAL for CBNAAT

BAL for CBNAAT	Liquid Culture		Total	p-value
	Negative	Positive		
Negative	21	7	28	0.001
	26.3%	8.8%	35.0%	
Positive	1	51	52	
	1.3%	63.8%	65.0%	
Total	22	58	80	
	27.5%	72.5%	100.0%	

The above table shows Correlations of liquid culture with BAL for CBNAAT. 58 study subjects were positive for liquid culture, out of which 51 subjects were positive and 7 subjects were negative for BAL for CBNAAT, 22 Subjects were negative for liquid culture in which 21 subjects were negative for BAL for CBNAAT, And 1 subject was positive for BAL for CBNAAT, on applying chi-square it was a significant association with p-value 0.001.

Table 5: Sensitivity and specificity of CBNAAT

Statistic for CBNAAT	Value (%)
Sensitivity	87.93
Specificity	95.45
Positive predictive value	98.07
Negative predictive value	75

The above table shows the Sensitivity and specificity of CBNAAT. The sensitivity of CBNAAT was 87.93%, specificity was 95.45%, positive predictive value 98.07%, Negative predictive value-75%.

ISSN 2515-8260 Volume 09, Issue 08, 2022

Discussion

The main bronchoscopic findings in our study were congestion with hyperemia, followed by mucoid to mucopurulent secretions, ulcerations, bleeding, intrabronchial growth and stenosis, which is consistent with a similar study conducted by Arshad Altaf Bachh *et al.* [5] where 70% had mucosa congestion, and in a similar study Kulpati *et al.* [6] observed the coating of the mucosa of involved segments with yellowish-white secretions. In 20% of patients, the segmental bronchus was constricted, and 20% of patients had ulceration.

In a similar study Purohit *et al.* [7] reported ulceration in 64% of patients; 60% had frothy secretion for the bronchus. Moderate hyperemia of bronchial mucosa was observed in all the patients.

Only 65 percent of the patients in the current investigation of bronchial washings to confirm tuberculosis diagnosis were positive for BAL for CBNAAT, while 35 percent of individuals were negative for BAL for CBNAAT with rifampicin resistance detected in two patients who had the previous h/o of PTB, confirmed by liquid culture. Similar results were seen with Afshan Ali Shaik *et al.* [8] with 66% of patients bronchial wash yielding positive results and in other studies, CBNAAT yielded 47.2 percent in a similar investigation by Sanjay awashiya *et al.* [9], and 44.6 percent in a study done by Panda RK *et al.* [10]. When compared to the latter two studies, our outcomes were superior, this may be due to selective patients who were referred by primary health centers and physicians after no improvement with initial treatment and who had worsened, to the TB clinic and as many of the patients were from the endemic areas surrounding davanagere.

In the current study, using solid/liquid culture as a gold standard, it was discovered that the sensitivity of CBNAAT was 87.93% and the specificity was 95.45%. In a similar study by Afshan Ali Shaik *et al.* [8] it was found to be 100% the specificity was 80% sensitivity. The positive predictive value was 47.8%, whereas the negative predictive value was 100%. Sensitivity and Specificity for CBNAAT were 82.3 percent and 98.5 percent, respectively, in research by Dr. Anuradha Chaudhary *et al.* [11] According to a research by Kalpesh Moradiya *et al.* [12], the sensitivity and specificity of CBNAAT for detecting MTB in lung samples of TB patients are 95.7 percent and 99.3 percent, respectively. The sensitivity and specificity of CBNAAT were reported to be 82.3 percent and 98.5 percent, respectively, in a study by Priti Singh *et al.*

Out of the total of 80 individuals enrolled in the trial, 22 had alternative non-TB diagnoses. Of the 22 non-TB conditions, 16 were diagnosed with bacterial pneumonia based on bacterial culture and sensitivity reports, two were proven to have bronchogenic carcinoma by endobronchial biopsy and CT guided FNAC/Biopsy and four were diagnosed with fungal pneumonia based on fungal culture and sensitivity reports.

Conclusion

This study demonstrates that CBNAAT is a simple tool that can aid in the rapid diagnosis of tuberculosis with high sensitivity, specificity, positive predictive value, and negative predictive value, with more than 90% sensitivity and 90-100 percent specificity. It can also detect rifampicin resistance at the same time, allowing appropriate and timely treatment to begin. It also aids in the detection of TB-negative patients, which contributes to cost savings

by avoiding unnecessary treatment.

References

1. Sukaguchi K, Yoneda T, Yoshikawa M, *et al*. Case study of interleukin-1 beta, tumor necrosis factor alpha and interleukin-6 production peripheral blood monocytes in patients with diabetes mellitus complicated by pulmonary tuberculosis [in Japanese]. Kekkaku. 1992;67(12):755-760
2. Shim WS, Kim SK, Kim HJ, *et al*. Decrement of postprandial insulin secretion determines the progressive nature of type-2 diabetes. Eur. J Endocrinol. 2006;155(4):615-622.
3. Stentz FB, Kitabchi AE. Transcriptome and proteome expressions involved in insulin resistance in muscle and activated T-lymphocytes of patients with type 2 diabetes. Genomics Proteomics Bioinformatics. 2007;5(3-4):216-235.
4. Sai S, Clemente-Casares X, Zhou AC, *et al*. Insulin receptor-mediated stimulation boosts T cell immunity during inflammation and infection. Cell Metab. 2018;28(6):922-934.e4.
5. Altaf Bachh, Arshad, *et al*. Diagnosing sputum/smear-negative pulmonary tuberculosis: Does fibre-optic bronchoscopy play a significant role? Lung India: official organ of Indian Chest Society. 2010;27(2):58-62. Doi: 10.4103/0970-2113.63607
6. Kulpati DD, Heera HS. Diagnosis of smear negative pulmonary tuberculosis by flexible fibreoptic bronchoscopy. Indian J Tuberc. 1986;33:179-82.
7. Purohit SD, Sisodia RS, Gupta PR, Sarkar SK, Sharma TN. Fibreoptic bronchoscopy in the diagnosis of smear negative pulmonary tuberculosis. Lung India. 1983;1:143-6.
8. Shaik AA, Kakodkar U, Borges C. Diagnostic Utility of Bronchial Washing CBNAAT for Mycobacterium Tuberculosis in Sputum Smear Negative and Sputum CBNAAT Negative Patients of Suspected Pulmonary Tuberculosis. International Journal of Innovative Research in Medical Science. 2021;6(03):201-204.
9. Avashia Sanjay, *et al*. To study the usefulness of CBNAAT (Cartridge Based Nuclear Acid Amplification Test) in BAL (Bronchoalveolar Lavage) samples in the diagnosis of smear-negative/non sputum producing patients with suspected tuberculosis. Journal of Evolution of Medical and Dental Sciences 5.1, 2016, 55-60.
10. Panda RK, Devi Jyoti Dash. To Study the Usefulness of Cartridge Based Nuclear Acid Amplification Test [CBNAAT] in Bronchoalveolar Samples in the Diagnosis of Sputum Negative Patients with Presumptive Pulmonary Tuberculosis. Int. J Pul. & Res Sci. 2019;4(2):555-632.
11. Chaudhary Anuradha. Comparing diagnostic accuracy of CB-NAAT and ZN staining in diagnosis of Pulmonary and Extra-pulmonary Tuberculosis cases at tertiary care teaching hospital of north India. Innovative Journal of Medical and Health Science, 2019, 9(636-645).
12. Moradiya Kalpesh, Muley Arti, Kumar Sunil, Patel Kinjal, Mahida Harshal. Cartridge based nucleic acid amplification test negative in highly suspected case of tuberculosis: a case report. International Journal of Research in Medical Sciences, 2020, 8. 10.18203/2320-6012.ijrms20200502.