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Original Research Article

Study the usefulness of cartridge based NUCLEIC acid amplification test in bronchoalveolar lavage samples in the diagnosis of smear-negative/non sputum producing patients with suspected tuberculosis

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ABSTRACT

Background: The aim of study is to evaluate the diagnostic value of the CBNAAT in BAL samples of smear negative for AFB or who could not produce an expectorated sputum sample.
Methods: A prospective and observational study of in patient and out patient department in Department of Pulmonary Medicine of Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar during the period November 2017 to November 2018.
Results: Bronchoalveolar lavage **c**artridge based nucleic acid amplification test in bronchoalveolar lavage was done for 60 samples of patients who were having history and chest x-ray suggestive of pulmonary tuberculosis with sputum AFB negative. Out of these sputum negative samples 25 were BAL CBNAAT positive and rest were negative.
Conclusions: CBNAAT adds significantly to the diagnostic yield of PTB in comparison to sputum smear microscopy. It has additional advantage of identifying rifampicin resistance with high sensitivity and specificity.

Keywords: Amplification test, Bronchoalveolar lavage, Pulmonary tuberculosis, Sputum negative

INTRODUCTION

Pulmonary tuberculosis is still one of the commonest cause of infectious disease related morbidity and mortality in the developing countries.[1] Diagnosis of pulmonary tuberculosis (PTB) mostly relies on identification of acid-fast bacilli (AFB) in sputum smear, but its limitation is low sensitivity.[2,3]

Conventional mycobacterial cultures (Solid culture in Lowenstein-Jensen medium) takes about 6-8 weeks' time newer liquid culture methods like BACTEC or Mycobacterial growth indicator tube (MGIT) gives relatively rapid results but is costly.[4,5] Cartridge based nucleic acid amplification test (CBNAAT) is a nested polymerase chain reaction (PCR) technique that identifies small quantities of genetic elements of Mycobacterium tuberculosis from clinical specimens and it can identify resistance to rifampicin, the surrogate marker of multi-drug resistant (MDR) tuberculosis, at the same time.

CBNAAT is completely automated, has minimal biosafety hazard and can give result within two hours. The purpose of our study is 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.

METHODS

A Hospital based prospective and observational study was carried out at Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar. Over a periods of 1-year from November 2017 to November 2018. In study period of 12 months among patients attending pulmonary medicine outpatient department and inpatient.

Clinico-radiologically suspected patients of pulmonary tuberculosis who were either sputum negative or not bringing out adequate sputum sample were included in the study. Included patients who do not have contraindications to bronchoscopy were subjected to the procedure and lavage fluid was obtained. Smear and CBNAAT examination of the fluid were done.

This study was reviewed and approved by Institutional Ethics Committee (IEC), Chalmeda Anand Rao Institute of Medical Sciences, Bommakal, Karimnagar. Informed oral consent was obtained from all the patients

Inclusion criteria

- Patients with clinical suspicion of PTB based on symptoms (e.g., cough more than two weeks
- Hemoptysis
- Fever
- Asthenia
- Loss of weight and night sweats) or
- Radiological features (e.g., nodule, consolidation, cavitation and other opacities) who either have a negative sputum AFB smear microscopy or were unable to produce sputum were included in the study.

Exclusion criteria

- Sputum positive cases,
- Isolated extra-pulmonary tuberculosis,
- HIV positive patients.

Patients not fit for bronchoscopy procedure, e.g. those having refractive hypoxemia, bleeding disorders, cardiovascular instability, status asthmaticus and marked hypercapnia were excluded from the study.

RESULTS

In our study 60, patients who were sputum negative suspected pulmonary tuberculosis underwent bronchoscopy. Out of 60 sputum negative BAL samples 25 (41.6%) were positive for CBNAAT and rest 35 (58.3%) samples negative. Among these 25BAL CBNAAT positive samples 22 were sensitive to rifampicin while 3 were resistance (Table 1).

In our study, patients aged between 18 to 50 years were 44 and remaining 16 patients were above 50 years. Most of the patients were above 50years.

Table 1: Demographic data.

Age	Male	Female	Total
18-50 years	28	16	44
>50years	10	6	16

In our study out of 38 male suspected tuberculosis patients 17 (44.7%) were CBNAAT positive and among 22 female suspected tuberculosis patients 8 (36.3%) were CBNAAT positive. Most of the male patients turned out to be positive for CBNAAT (Table 2).

Table 2: CBNAAT results.

	CBNAAT positive	CBNAAT negative	Total
Male	17	21	38
Female	8	14	**22**
Total	25	35	60

Table 3: Results of tests.

Results	Frequency	Percentage
Total Cases	60	100
Bal Cbnaat Negative	35	58.3
Bal Cbnaat Positive	25	41.6
Rifampicin Sensitive	22	36.6
Rifampicin Resistance	03	5

Table 3 shows the results of the tests, out of 60 BAL samples of suspected tuberculosis patients, 35 (58.3%) samples were CBNAAT negative and 25 (41.6%) were samples were CBNAAT positive. Out of 25 CBNAAT positive samples 3 (5%) samples were rifampicin resistant.

DISCUSSION

Sputum negative pulmonary tuberculosis constitutes about 50% of all new cases of pulmonary tuberculosis. Although the relative transmission rate of smear negative tuberculosis is lower than that of smear positive cases, it is still responsible for 17% of tuberculosis transmission.[6]

Conventional laboratory techniques like direct microscopy are less sensitive and going for culture is a time consuming process for the diagnosis of tuberculosis. Therefore, it is the need of time to develop new techniques for rapid identification of the Mycobacterium tuberculosis in pauci-bacillary samples. Recently, attention has been devoted to latest nucleic acid amplification diagnostic processes due to their speed and accuracy.

Several polymerase chain reaction-(PCR) based molecular methods have recently been developed for early TB diagnosis and rapid detection of drug resistance from clinical specimens.[7,8] The CBNAAT (Cartridge based nuclear acid amplification test) is one of these

methods, and consists of a hemi-nested real-time PCR test that simultaneously identifies Mycobacterium tuberculosis and detects rifampicin resistance, as a surrogate of multidrug resistance (MDR), directly from clinical specimens. Since December 2010, WHO has recommended the CBNAAT as a bona fide test due to its high-quality performance as compare to microscopy especially in cases of smear-negative cases.[9]

Moure R et al in their research in 2012 concluded that out of 108 smear-negative extrapulmonary samples 58.3% were positive with the Xpert MTB/RIF assay (GX) for Mycobacterium tuberculosis.[13] In a similar study by Vadwai in 2011, the sensitivity of the Xpert assay was 64% for smear-negative cases.[14] Our results are little lower (41.6%) than these two study as we had not performed CBNAAT on BAL smear AFB positive samples and our sample size was much less as compared to other studies.

In a study conducted by Mohanty T et al shows out of 71 patients who were smear AFB-negative cases, BAL for CBNAAT was positive in 23 (32%) patients.[12] In our study out of 60 BAL samples of suspected tuberculosis patients, 35 (58.3%) samples were CBNAAT negative and 25 (41.6%) were samples were CBNAAT positive. Out of 25 CBNAAT positive samples 3 (5%) samples were rifampicin resistant.

CONCLUSION

The results of the study revealed a maximum positivity rate by CNAAT, which indicated that it is more sensitive technique as compared to conventional methods. We found out 25 samples were positive and 3 were resistance to rifampicin which we could have missed with AFB staining. Its simplicity, sensitivity, speed and automation make this technique a very attractive tool for diagnosis of mycobacterial tuberculosis from smear negative cases of TB suspects.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee of Chalmeda Anand Rao Institute of Medical Sciences, Bommakal, Karimnagar, Telangana, India

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