Original Research Article

A study on early detection of pulmonary tuberculosis in smear negative retroviral positive patients by using CBNAAT

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International Archives of Integrated Medicine, Vol. 6, Issue 3, March, 2019.

Available online at http://iaimjournal.com/
ISSN: 2394-0026 (P) **ISSN: 2394-0034 (O)**
Received on: 25-02-2019 **Accepted on:** 01-03-2019
Source of support: Nil **Conflict of interest:** None declared.
How to cite this article: V. Rajkumar, Karthikeyan. A study on early detection of pulmonary tuberculosis in smear negative retroviral positive patients by using CBNAAT. IAIM, 2019; 6(3): 349-354.

Abstract

Background: Tuberculosis is one of the most common opportunistic infections among people with HIV infection. Detection of pulmonary tuberculosis by sputum-based techniques includes microscopy and culture. However, in people living with HIV, sputum production is scanty and also the sputum contains less number of bacilli due to fewer cavitations, thereby decreasing the sensitivity and specificity of sputum microscopy as a diagnostic tool.

Aim of the study: In this study, we assess the usefulness of CBNAAT in the early detection of pulmonary tuberculosis and its incidence by using CBNAAT in smear-negative HIV patients using mycobacterial culture in Lowenstein Jensen medium as Gold Standard.

Materials and methods: The study was conducted in the Department of Cardiothoracic Surgery, Government Mohan Kumaramangalam Medical College Hospital. Data were collected from 150 HIV infected patients who tested sputum smear negative. Sputum samples were then sent for CBNAAT and sputum culture for mycobacteria.

Results: Of the 150 patients enrolled, 28(18.66%) of them were detected with MTB by CBNAAT; whereas sputum culture could detect 38(25.33%) of them. Thus, compared to sputum smear, CBNAAT increases TB detection by 18.66% and sputum culture increases by 25.33%. The sensitivity of CBNAAT in our study was 73.68% and the incidence of smear-negative pulmonary TB in the study population by using CBNAAT was 18.66%.

Conclusion: CBNAAT is a highly sensitive and diagnostic stool for the diagnosis of pulmonary TB and it is of immense help in the early diagnosis of smear-negative pulmonary TB in HIV infected patients. Therefore, CBNAAT should be used as the initial test in HIV infected patients suspected with pulmonary TB.

Key words

Cartridge-based nucleic acid amplification test (CBNAAT), HIV infection, Pulmonary tuberculosis.

Introduction

Tuberculosis is an infectious disease caused by a bacterium called Mycobacterium tuberculosis. Tuberculosis mostly involves the lungs; however, it can also affect other organs in the body [1]. Tuberculosis is one of the most common opportunistic infections amongst people with HIV and TB-HIV co-infection even further increases the mortality of an individual [2]. The clinical presentation of pulmonary tuberculosis depends on the immune status of an individual and an immune-suppressed individual with HIV can present with atypical features, thus posing diagnostic challenges [3]. Detection of pulmonary tuberculosis by sputum-based techniques includes microscopy and culture. However, in people living with HIV, sputum production is scanty and the also the sputum contains less number of bacilli due to fewer cavitations, thereby decreasing the sensitivity and specificity of sputum microscopy as a diagnostic tool [4]. To overcome these shortcomings, mycobacterial culture is an alternative. However, it is a time-consuming technique which can take 4-8 weeks for the result thereby causing a delay in early initiation of anti-tubercular drugs, increasing the risk of transmission to close contacts and also the spread to extrapulmonary regions within the same individual [5]. The Xpert MTB/RIF is a cartridge-based nucleic acid amplification test, an automated diagnostic test capable of identifying Mycobacterium tuberculosis DNA and rifampicin resistance by nucleic acid amplification in real time. CBNAAT can detect as few as 131 CFU/ml of MTB whereas sputum microscopy has a limit of detection of ~10,000 CFU/ml of MTB. Xpert MTB/RIF is the initial diagnostic test to detect pulmonary TB and rifampicin resistance in all patients with signs and symptoms of TB as recommended by WHO [6].

Materials and methods

The study was conducted in the Department of Cardiothoracic Surgery, Government Mohan Kumaramangalam Medical College Hospital. Data were collected from 150 HIV infected patients who tested sputum smear negative. Sputum samples were then sent for CBNAAT and sputum culture for mycobacteria.

Inclusion criteria

- All retroviral positive patients (Newly detected + Old) without a history of TB.
- Retroviral positive patients with Negative Sputum Smear.

Exclusion criteria

- Retroviral negative patients.
- Retroviral positive patients with positive sputum smear.
- Patients less than 13 years.

Sample collection

Two-morning sputum samples were collected for each patient and then sent for sputum microscopy. Another sample was also collected and then sent for mycobacterial culture. Sputum smears negative for AFB were again sent for CBNAAT.

Method of testing

Sputum AFB – Ziehel Neelsen stain and Light Microscopy. Nucleic acid amplification test – CBNAAT (Cartridge Based Nucleic Acid Amplification Test).sputum culture – Agar based solid Lowenstein Jensen (LJ) Medium.

Statistical analysis

The collected data were analyzed with IBM.SPSS statistics software 23.0 version. To describe about the data descriptive statistics frequency analysis, percentage analysis was used for categorical variables and the mean & S.D were used for continuous variables. The Sensitivity was used to find the efficacy of CBNAAT with Sputum culture findings and also

the incidence of TB was determined from the variables.

Result interpretations

Sputum AFB +ve: Pulmonary TB present.

Sputum AFB –ve: The case was negative for sputum AFB and further evaluated with CBNAAT and mycobacterium culture.

CBNAAT: MTB detected – Pulmonary TB present.

Sputum culture in LJ medium: Brown and granular colonies have grown – MTB has grown – Pulmonary TB present.

Results

Female patients were 58% and males were 42%. Females were more in our study population when compared to males (**Table – 1**). Sensitivity of CBNAAT= 73.68% (**Table – 2**). Incidence rate as per CBNAAT- 18.66%, Incidence rate as per sputum culture = 25.33% (**Graph – 1**).

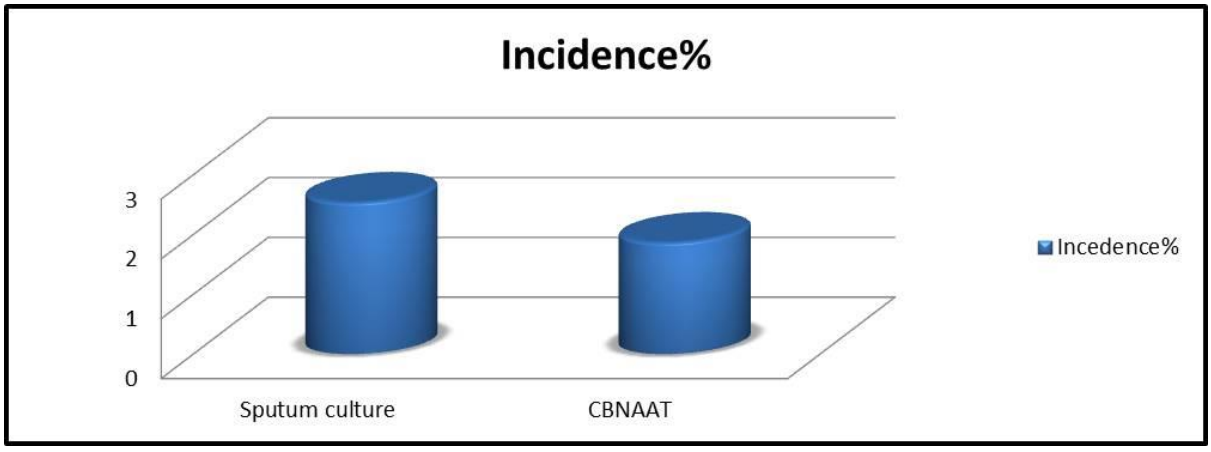
Table – 1: Gender distribution in the study population.

Gender	Frequency	Percentage
Male	63	42
Female	87	58
Total	150	150

Table – 2: Cases detected by CBNAAT and sputum culture.

		SPUTUM CULTURE		Total
		MTB GROWN	MTB NOT GROWN	
CBNAAT	Detected	28(A)	0(B)	38
	Not detected	10(C)	112(D)	122
Total		38	112	150

Graph – 1: Incidence rate of pulmonary TB in smear negative HIV patients separately for sputum culture and CBNAAT.



Discussion

In TB patients with HIV co-infection, the sputum is often scanty and the bacillary content of the sputum is also due to frequent absence of cavitary lesions. Consequently, these result in decreased sensitivity and specificity of sputum microscopy as a diagnostic tool [7]. The conventional gold standard test, sputum culture for Mycobacterium, is unsuitable for screening purposes as it is slow and time-consuming, taking 4-8 weeks, and also a reliable testing facility not easily and widely available [8]. Since the delay in initiation of anti-tubercular treatment can lead to an increase in the risk of transmission

of TB in the community and close contacts and also its spread to extrapulmonary regions within the patient, there is an urgent need to have a diagnostic tool which will be fast enough to provide results within few days and with a high degree of sensitivity and specificity [9]. Gene Xpert/MTB-RIF also known as cartridge-based nucleic acid amplification test (CBNAAT) which is highly sensitive and specific for *Mycobacterium tuberculosis*, has been recently introduced for detection of TB. It has an added advantage to detect rifampicin resistance [10]. In our study, we assess the diagnostic sensitivity of CBNAAT using sputum culture as the Gold Standard in smear-negative HIV infected patients. We also determine the incidence of pulmonary TB in the same study population [11]. Our study showed that of the 150 patients enrolled over a period of one year, a total of 38 patients were detected with Mycobacterium tuberculosis by sputum culture, out of which CBNAAT could not detect 10 of them(38 vs 28). This shows that CBNAAT helps in early diagnosis of pulmonary tuberculosis in smear-negative HIV patients by about 18.6 percent [12]. Using sputum culture as the gold standard, the sensitivity of CBNAAT in our study was 73.68%. By cross-sectional analysis, the incidence of pulmonary tuberculosis in HIV infected patients in our study population using CBNAAT and sputum culture were 16.88% and 25.33% respectively. These findings are in accordance with many studies carried out by various scholars in other parts of the world [13]. Toossi Z, et al., in multicenter cross-sectional study observed that the Xpert MTB/RIF detected 89% of TB cases with high specificity (99%) when used as an initial screening test. As an add-on test following smear microscopy, Xpert MTB/RIF detected 67% of TB cases with high specificity (99%). Sensitivity for smear-positive, culture-positive TB was 98% for Xpert-MTB/RIF. The GeneXpert/MTB/RIF increased TB detection in culture-confirmed cases by 23%. It also detected 79% of pulmonary TB cases in people infected with HIV and 86% of pulmonary TB cases in people without HIV (99). Among people with HIV, Xpert MTB/RIF pooled sensitivity was 61% (95% CrI 40% to 81%) for smear-negative, culture-positive TB compared with 97% (95% CrI 90% to 99%) for smear-positive, culture-positive TB, which was statistically significant [14]. Srikantiah P Peter et al.in their study titled "Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults" observed that Xpert MTB/RIF diagnosed 47% (95% CI, 33–61%) of smear-negative TB cases in a high HIV prevalence setting and 55% (35–73%) when a restricted analysis was carried out [15]. Boehme Meintjes G, et al., observed that in culture-positive patients, a single, direct MTB/RIF test identified 551 of 561 patients with smear-positive tuberculosis (98.2%) and 124 of 171 patients with smear-negative tuberculosis (72.5%) [16]. Lawn SD, et al., in their study reported that the overall sensitivity of the Xpert MTB/RIF assay for culture-positive TB was 73.3% compared to 28.0% using smear microscopy. All smear-positive, culture-positive disease was detected by Xpert MTB/RIF from a single sample (sensitivity, 100%), whereas the sensitivity for smear-negative, culture-positive TB was 43.4% and 62.3 % from one sputum sample and two sputum samples respectively [17, 18].

Conclusions

Significant proportion of patients having pulmonary tuberculosis with HIV co-infection is smear-negative for acid-fast bacilli. There is a high incidence of tuberculosis among people living with HIV. Sputum microscopy is not a sensitive diagnostic tool for diagnosing pulmonary tuberculosis in people living with HIV. CBNAAT is a highly sensitive and specific tool for the diagnosis of TB and it is highly useful for the early diagnosis of smear-negative TB in HIV infected patients.

Acknowledgments

Authors would like to thank the General Medicine Department faculty, Govt. Mohan Kumaramangalam Medical College, Salem for

their humble support to complete the research work.

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