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Comparison of fluorescent stain and CBNAAT in sputum sample for the diagnosis of pulmonary tuberculosis

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Abstract
Tuberculosis caused by Mycobacterium Tuberculosis is very common infectious diseases in India primarily affecting lungs. Various diagnostic modalities are available at present for diagnosis of tuberculosis. CBNAAT (cartridge based nucleic acid amplification test) is a newer modality recommended by World Health Organization. It is very sensitive method and can detect even if few bacilli are present in the sample. Fluorescent microscopy is also very sensitive method and need a fluorescent microscope. As in our study out of 120 patients, five patients were positive only in CBNAAT while four patients were positive only in fluorescent staining. This indicates that fluorescent staining method and CBNAAT should be used together for the early detection of pulmonary tuberculosis.

Keywords: Tuberculosis, CBNAAT, Fluorescent microscopy

Introduction

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis. In 2018, approximately 10 million people fell ill with TB. Globally there were 1.2 million TB deaths among HIV negative people in 2018 and an additional 251000 deaths among HIV-positive people. (1). Pulmonary tuberculosis should be suspected in patients with relevant clinical manifestations of coughing >2 to 3 weeks duration, fever, weight loss, night sweats and cervical lymphadenopathy. Various investigations can be used to help in the diagnosis of tuberculosis, and these include chest radiographs, clinical suspicion, staining technique, culture for mycobacterium, and nucleic acid amplification assays. Sputum smear microscopy is the preferred and rapid test that is widely used for the detection and diagnosis of pulmonary tuberculosis [2, 3]. The bacilli in the sputum can be detected either by ZN or fluorescence staining techniques. However bacilli can be missed in smear examination if they are only few in numbers. In that case CBNAAT method and culture are helpful. CBNAAT can detect even if few bacilli are present in sample, simultaneously it also detects the Rifampicin resistance. Culture is gold standard method for the diagnosis of TB; drug sensitivity can also be detected further if the culture samples are positive for TB. As per Revised National Tuberculosis Control Program (RNTCP) or World Health Organization (WHO), an individual with at least one sputum smear positive for AFB or culture positive for tubercle bacilli is labeled to be suffering from pulmonary tuberculosis. WHO also recommended a Real-time PCR test (CBNAAT) as a primary diagnostic modality [4, 5].

Material and Methods

This retrospective study was carried out in pathology department at school of excellence in pulmonary medicine Netaji Subhash Chandra Bose medical college Jabalpur. The data of 120 patients were collected from 1st February to 29th February who presented in the OPD with the symptoms of tuberculosis. The patients were requested to submit two sputum samples each in a clean, sterile, leak-proof, wide-mouth containers. One sample from each patient was taken on the spot and the patients were provided with a second pre labeled container for a morning sample to be taken at home. Patients are instructed on the difference between sputum and saliva. For each sample, the smears were made in duplicate and were

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subjected for fluorescence staining. While CBNAAT testing was carried out in only morning samples as the chances of getting bacilli is more in morning samples. Inclusion criteria was all new patients of both gender suspected to be a case of pulmonary tuberculosis as per RNTCP guidelines while the exclusion criteria was samples macroscopically resembling saliva were excluded from the study.

Fluorescence Microscopy Staining Procedure: The smears were flooded with filtered 0.1% auramine for at least 20 minutes. They were then rinsed with water and drained. Acid alcohol decolorizing solution (0.5%) was applied on the smear for 30 to 60 seconds, rinsed with water, and drained. They were then flooded with 0.5% potassium permanganate counter stain for a maximum of 1 minute and rinsed with water. The smears were allowed to air dry and examined microscopically using the dry (40x) objective lens of an LED illumination-based fluorescence microscope.

Xpert MTB/RIF Assay Procedure: Cartridge based nucleic acid amplification test (CBNAAT) is a newly introduced method for detection of TB which is based on polymearse chain reaction. The XpertMTB/ RIF assay was performed following the protocol of the manufacturer (Cepheid Gene Xpert). Samples were collected in containers provided and treated with sample reagent in a proportion of 2:1 and incubated for 15 minutes at room temperature. Two milliliters (2 ml) of the reagent treated sample was pipetted into the sample chamber of the Xpert cartridge. The Xpert cartridge was then placed into the GeneXpert instrument system and run. Results were generated within 120 minute. *The* XpertMTB/ RIF assay simultaneously detects Rifampicin resistance by amplifying a MTB complex specific sequence of the rpoB gene which is probed with five molecular beacons for mutation within the rifampicin resistance determining region.

Results
In our study, sputum samples of 120 patients were evaluated for fluorescent stain and CBNAAT. Out of 120 patients 60 patients were positive for TB either in fluorescent stain or in CBNAAT or in both, while remaining 60 patients were negative for TB. Out Of 60 positive patients, 51 patients were positive in both fluorescent stain and CBNAAT, 04 patients were positive only in fluorescent stain while 05 patients were positive only in CBNAAT. Out of 55 patients which were positive in fluorescent stain 53 were positive in both spot and morning samples while in 2 patients spot sample was negative and morning sample was positive. Also in fluorescent stain positive patients the grading of positivity was higher in morning samples as compare to spot samples. Thus the smear positivity rate of fluorescent stain was 45.83%, while the detection of MTB in CBNAAT was 46.66%.

Discussion
Tuberculosis is major health problem in developing countries. Various diagnostic modalities are available for diagnosing TB of which the staining method is fastest, cheapest and reliable method. Fluorescent stain has been added in Revised National Tuberculosis Control Program (RNTCP) because of more sensitive and rapid results and can be used in field areas. In this retrospective study, we

have evaluated the diagnostic yield of Gene Xpert and Fluorescent stain to detect MTB in sputum samples and compared them with each other. In this study, the results showed that from sputum specimen of 120 patients, 55 patients were smear positive with fluorescent stain and 56 were positive in CBNAAT. These results indicate that CBNAAT is slightly more sensitive for the diagnosis of TB and difference in rate of positivity by both methods is minor. The Auramine staining of sputum smears is almost comparable with CBNAAT. Fluorescent staining method is economical in both time and cost and recommended for laboratories handling large number of sputum specimens and can also be used in periphery/field areas where CBNAAT method is not available for the diagnosis of TB. However CBNAAT is more effective in diagnosing the TB. Numbers of studies have demonstrated the utility of GeneXpert in diagnosis of pulmonary tuberculosis [6-9].

Conclusion
CBNAAT is more effective in detection of pulmonary TB. It has an additional feature of detecting rifampicin resistance. As in our study five patients were positive only in CBNAAT while four patients were positive only in fluorescent staining. This indicates that fluorescent staining method and CBNAAT should be used together for the early detection of pulmonary tuberculosis.

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