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Original Research Article

Role of GeneXpert in the Diagnosis of Multi Drug Resistant Tuberculosis

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ABSTRACT

India is one of the largest contributors to the global burden of tuberculosis. The detection of extra pulmonary TB and pauci-bacillary nature of the specimen poses a challenge for its diagnosis. WHO has recommended GeneXpert assay as a rapid diagnostic test for detection of tubercle bacilli and the presence of Rifampicin resistance. In this study we assess the efficiency of GeneXpert in diagnosis of TB in comparison with standard microscopy test and also its role in detection of MDR TB. Out of1091 patients who were clinically suspected of suffering from tuberculosis 380 (i.e. 34.83%) were positive on GeneXpert for detection of MTB complex and only19.31% (i.e. 62) were positive by conventional routine ZN microscopy. Out of these TB positive patients 61 were infected by MDR strains. Our study shows that the GeneXpert MTB/RIF assay is a useful tool for rapid diagnosis of pulmonary and extra pulmonary tuberculosis as it has greatly shortened the time of detection up to two hours.

Keywords: - GeneXpert, multi drug resistant tuberculosis, Extra pulmonary TB

INTRODUCTION

India is one of the largest contributors to the global burden of tuberculosis. In the ancient times it was also referred to as 'phthisis' or 'The White Plague'. The causative agent, *Mycobacterium tuberculosis* is a potentially infectious organism with high incidence of mortality and morbidity. According to WHO statistics in 2016, 10.4 million new TB cases were estimated and only 6.3 million newly diagnosed TB cases were actually notified. [(1)] Despite the high load of tuberculosis in the world, not every case is reported to the healthcare system. Also, there is a wide spread emergence of MDR/TB and XDR/TB strains. This could be attributed to the lack of effective, rapid, user-friendly, point-of-care tests. To help eliminate this highly infectious disease, rapid diagnosis, patient awareness and adherence to treatment will form the cornerstone in the End TB-2025 campaign by The Government of India. Hence WHO has recommended GeneXpert assay as a rapid diagnostic test for detection of tubercle bacilli and the presence of Rifampicin resistance. [(1)]

Tuberculosis mainly manifests as a pulmonary disease. Epidemiological data suggests the incidence of pulmonary disease to be 80-85% while that of extrapulmonary to be 15-20% of all the tuberculosis cases. However, in HIV coinfected patients' extrapulmonary TB accounts for around 50% of the total HIV/TB coinfected patients. [(2)] For diagnosis of pulmonary TB early morning expectorated sputum samples

are preferred. The detection of extra pulmonary TB and paucibacillary nature of the specimen poses a challenge for its diagnosis. Microscopy has a low sensitivity in diagnosis of acid fast bacilli in the smear, which gives a high false negative result. Also, the gold standard tests like solid/ liquid culture media require a longer turn-around time (average of 3-8 weeks) for confirmation and it requires well trained laboratory personnel. Delay in effective diagnosis can cause more harm to the patient and the society due to the infectious nature of this organism.

Aim & Objective:

- To compare the sensitivity of Ziehl Neelson staining with GeneXpert assay.
- To assess the role of GeneXpert in diagnosis of multi drug resistant tuberculosis.

MATERIALS & METHODS

This is a prospective study carried out on all samples received from January 2018 to June 2018 in the Tuberculosis laboratory, Department of Microbiology, MGM Medical College and Hospital, Navi Mumbai, India. Samples were collected from patients clinically suspected of tuberculosis, previously treated patients, patients with history of contact with drug resistance visiting DOTS centre of MGM Medical Hospital and key population. Sputum samples were processed for smear microscopy for detection of acid fast bacilli by ZN staining and GeneXpert MTB/RIF assay. MDR-TB is defined as tuberculosis which is resistant to Rifampicin and/or Isoniazid. GeneXpert MTB/RIF assay is a cartridge based nucleic acid amplification test based on the principle of semi-nested Real Time PCR. It is specific for the detection of DNA of *Mycobacterium tuberculosis* and diagnosis of resistance to Rifampicin (i.e. detection of mutation in rpo-β gene).

The target is the rpo-β gene (3 primers and 5 probes) which belongs to the conserved region of the MTB complex. These probes can differentiate between conserved wild- type sequence and mutations in the core region that is associated with Rifampicin resistance.

Procedure for preparation of smears for ZN staining and processing of the samples of GeneXpert MTB/RIF assay were carried out in a biosafety cabinet under biosafety level IIB protocols.

ZN staining Procedure:

A heat fixed air-dried smear was made from the sample and stained with Carbol fuschin as the primary stain with intermittent heating, 25% H_2SO_4 as the decolouriser (according to RNTCP protocols) and Methylene blue as the secondary stain. The above-mentioned smear was examined under oil immersion lens and a minimum of 200 fields were scanned before declaring the smear negative for presence of acid fast bacilli. Presence of mycolic acids, glycerol and other fatty acids in the cell wall confers the acid fastness to the organism. Acid fast bacilli will take up the primary stain and appear as pink rod-shaped organisms.

GeneXpert MTB/RIF Assay Procedure:

Specimen on which the test is to be conducted is collected in a 50 ml falcon tube. Sample liquefaction is done with the sample reagent in the ratio of 2:1 (Reagent: Specimen). This mixture is vortexed and then incubated at room temperature for 10minutes. Mix well again and incubate it further for 5 more minutes at room temperature. Liquefaction of the sample is ensured by slightly tilting the tube and seeing that the sample doesn't stick to the tube walls. 2 ml of this mixture is then transferred into the GeneXpert cartridge. Sample is then loaded into the GeneXpert machine. Within the machine there is ultrasonic lysis of filtered captured organisms to release the DNA which is then mixed with dry PCR reagents. It then proceeds to a semi-nested real time amplification and detection of MTB complex in the specimen. [(3)]

Inclusion& Exclusion Criteria:

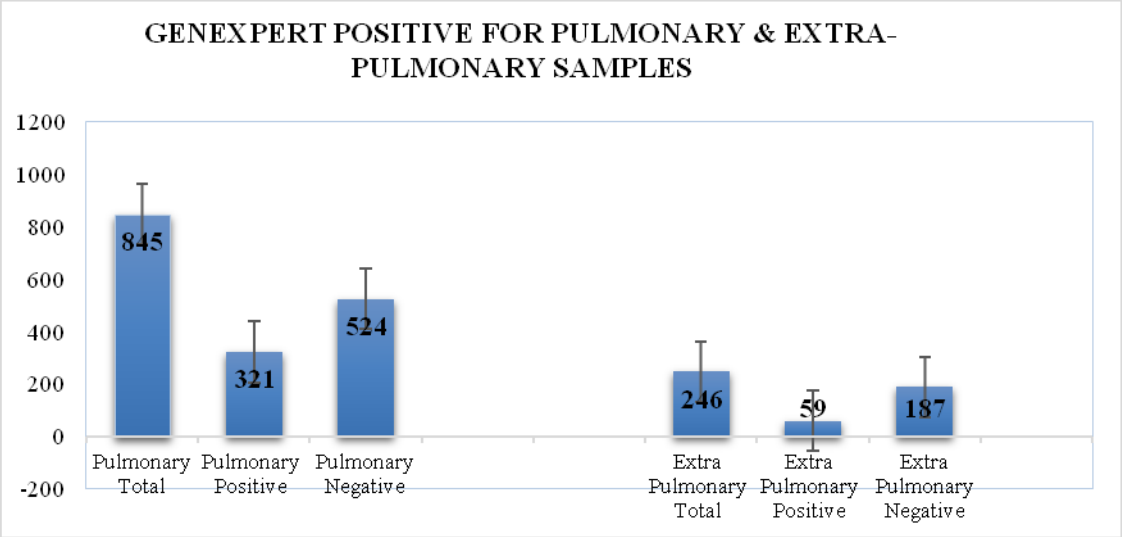
All the samples of patients clinically suspected of having pulmonary / extra pulmonary tuberculosis were included in

our study. Productive sputum samples collected in falcon tube were accepted for testing. For extra pulmonary samples, the samples collected with all aseptic precautions in falcon tube and transported immediately to the laboratory were included.

Salivary samples of sputum were excluded from our study. Samples containing food particles/ tobacco were also excluded from our study.

RESULTS AND DISCUSSION

A total of 1091 patients who were clinically suspected of suffering from tuberculosis were subjected for GeneXpert testing. Out of these, 380 (i.e. 34.83%) were positive on GeneXpert for detection of MTB complex.

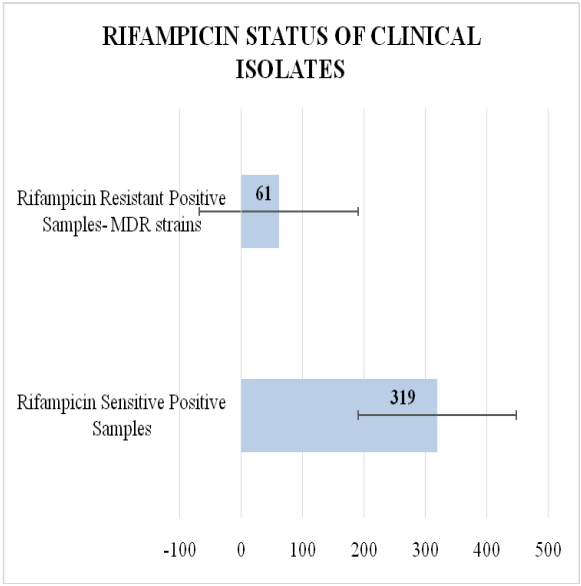


Out of the total 845 pulmonary samples, 321 (37.99%) pulmonary samples were positive for detection of *M. tuberculosis.* Whereas, out of the 246 extra pulmonary samples, 59 (i.e. 23.98%) were positive for detection of MTB complex.

In a similar study conducted by Arzu N. Zeka et al, a total of 429 samples (253 pulmonary and 176 extrapulmonary) were subjected for GeneXpert out of which 77 were positive for MTB. 17% of total pulmonary samples were positive for MTB, while 19.31% of total extra pulmonary samples were positive for MTB. [(4)]

Another study done by Souad M. Al-Ateah el al, a total of 239 samples were subjected to GeneXpert of which 59 (24.68%)were positive. 42 out of 172 pulmonary samples (24.42%) were positive for MTB whereas 17 out of 67 were extra pulmonary samples (25.37%) were positive. [(5)]

Newly diagnosed cases with clinical suspicion of tuberculosis were 247 out of the total samples screened (i.e. 22.64%). Whereas, 133out of the total patients (i.e. 12.19%) screened gave a history that they were on AKT therapy in the past.

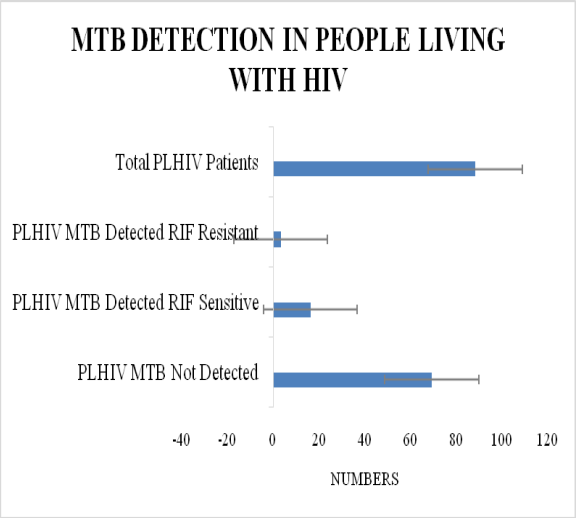


Amongst the GeneXpert positive cases, 83.95% were sensitive to Rifampicin. Whereas 16.05% were resistant to Rifampicin drug i.e. were MDR strains.

Majority of the MDR patients belonged to the category who gave a past history of anti-tubercular treatment in the past.

SWELLING/ ABSCESS ASPIRATE	LYMPH NODE	PUS	PLEURAL FLUID	ANKLE TISSUE	FNAC	CSF	GASTRIC ASPIRATE
5	33	4	11	1	3	1	1

Amongst the sites for extra pulmonary tuberculosis positive patients, maximum positivity rate was form lymph node biopsy specimens, followed by pleural fluid. In our study setting we came across only one established case of tuberculous meningitis which was confirmed on GeneXpert.



Amongst the target population i.e. PLHIV patients, 5% of the total positive patients for MTB complex belonged to PLHIV group. 2.93% of the total screened population was specific to target population i.e. the pediatric age group. Amongst the people detected with MTB complex only 1.05% belonged to the pediatric population.

Out of the total positive pulmonary samples subjected to GeneXpert, only 19.31 (i.e. 62) were positive by conventional routine ZN microscopy. Out of this 1 sample was positive by ZN microscopy but negative on GeneXpert. Further tests on that sample showed it belonged to the group of non-tuberculous mycobacteria. Hence, establishing the fact that GeneXpert is specific only to the diagnosis of *M. tuberculosis*.

In our study, we found that 16.05% of the total positive isolates were found to be MDR strains on GeneXpert assay testing. In a study conducted by Sauzullo et al, 3.44% of the positive specimens were found out to be MDR strains. [6]

In a study conducted by Monika Agarwal et al [7] 24.70% of the total samples screened were found to be MTB positive by GeneXpert assay. Also 1-7% of the total samples screened were found to be MOTT strains.

CONCLUSION

Tuberculosis from clinical specimens is less sensitive as compared to the culture-based techniques, because large bacillary load will be required for a smear to become positive. Moreover, the conventional culture-based systems are time consuming, require trained laboratory personnel. The GeneXpert MTB/RIF assay is a rapid molecular biology-based assay that can be used close to the point of care by operators with minimum technical expertise. The technique enables quick diagnosis of TB. The extra advantage is the convenience of sample processing where unprocessed sputum samples, as well as clinical specimen from extra pulmonary sites, can be directly assayed. Our study shows that the GeneXpert MTB/RIF assay is a useful tool for rapid diagnosis of pulmonary and extra pulmonary tuberculosis as it has greatly shortened the time of detection up to two hours. GeneXpert is specific only to the diagnosis of *M. tuberculosis and eliminates MOTT*. This method could offer a new technique though expensive, but quicker and more specific.

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