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COMPARATIVE STUDY OF GENEXPERT WITH ZN STAINING IN SPUTUM SAMPLES OF SUSPECTED PULMONARY TUBERCULOSIS

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ABSTRACT

Background: Tuberculosis remains one of the deadliest communicable diseases. There are number of tests available for the diagnosis of tuberculosis but conventional microscopy has low sensitivity and culture although gold standard, takes longer time for positivity. Gene-Xpert is real-time PCR based rapid molecular assay for diagnosing TB. By early detection of tuberculosis, GeneXpert can prevent the spread of TB. **Aim:** The aim of this study was to find the effectiveness of GeneXpert in detecting the MTB in sputum samples of suspected pulmonary tuberculosis patients and to compared with ZN staining (AFB smear microscopy). **Materials and Methods:** We retrospectively reviewed the sputum samples of suspected pulmonary tuberculosis 98 patients for 2 years. The sensitivity, specificity, PPV and NPV of GeneXpert and ZN microscopy were calculated using Mycobacterium tuberculosis culture as gold standard. **Results:** A total of 98 patient samples were evaluated in final analysis. Of these, 66 sputum samples were negative by all three methods used. 21sputum samples were ZN staining positive & 31 samples (31.6%) were GeneXpert TB positive. Out of 77 ZN staining negatives samples, 10 were positive in GeneXpert. The overall sensitivity, specificity, PPV and NPV of GeneXpert were 96.8%, 100%, 100%, & 98.5% respectively. **Conclusion:** GeneXpert is more accurate and reliable than sputum smear microscopy. GeneXpert can be a useful tool for early diagnosis of patients with high clinical suspicion of pulmonary tuberculosis.

KEYWORDS: Pulmonary TB, Gene Xpert, Rapid diagnosis.

INTRODUCTION

According to the Global Tuberculosis report of World Health Organization (WHO), Tuberculosis (TB) remains one of the world's deadliest communicable diseases that is caused by the Bacterium *Mycobacterium tuberculosis* (MTB).[1] In May, 2012 India declared TB a notifiable disease. Out of the estimated global annual incidence of TB cases, India shares one fourth of the cases.[2] The disease usually spread by air transmission from people with pulmonary TB.

Being rapid yielding, simple, Sputum Microscopy (SM) has been the main diagnostic tool, followed by sputum culture, the 'gold standard'. False-negative results and misdiagnosis of TB suspects are common in developing countries, as most TB control programmes use Ziehl-Neelsen (ZN) smear microscopy, which has poor sensitivity and multiple visits are required that leads to higher default. Mycobacterial culture is slow and usually takes 2-6 weeks time to get a final result and requires technical expertise and proper infrastructure.[3,4]

Accurate diagnosis of all cases are required to control TB and can only be achieved through affordable newer diagnostic tools. It may reduce the direct costs of diagnostic burden on patients and their families and also help national TB control programs to start early treatment. Early diagnosis is imperative for early patient management and successful outcomes.

There are number of Nucleic Acid Amplification (NAA) methods that have been developed for rapid identification of *Mycobacterium tuberculosis* (MTB) in clinical specimens of pulmonary and extra-pulmonary tuberculosis cases.[5-7] These techniques not only provide the advantage of rapidity of diagnosis but also detect even low MTB genomic copies in various specimens.

WHO endorsed the GeneXpert for the diagnosis of TB.[5] The GeneXpert utilizes a DNA-PCR technique for simultaneous detection of *Mycobacterium tuberculosis* and Rifampicin resistance related mutations. It is the first fully automated bench top cartridge based nucleic acid amplification (CB-NAAT) assay for TB detection that includes all necessary steps of DNA PCR. It gives results within 2 hours. Diagnostic accuracy of GeneXpert for pulmonary TB has been reported high.[8,9]

RNTCP is currently using GeneXpert to diagnose Pulmonary TB, Paediatric TB, Extrapulmonary TB, Rifampicin resistance and MDRTB in high risk populations like HIV positives as recommended by WHO under 2013 policy recommendations.[10-12]

Aim

The aim of this study was to find the effectiveness of GeneXpert in detecting the MTB in sputum samples of suspected pulmonary tuberculosis patients and to compared with ZN staining (AFB smear microscopy).

MATERIALS AND METHODS

Inclusion criteria

Patients with clinical suspicion of pulmonary tuberculosis including symptoms of cough with or without expectoration for >2 weeks, weight loss, fatigue, haemoptysis and loss of appetite.

Exclusion Criteria

Samples received without clinical history & Patient with history of lung malignancies or fungal infectionswere excluded from this study.

Sputum of 98 patients with suspected pulmonary tuberculosis, received retrospectively for the request of AFB smear microscopy(ZN staining) and GeneXpert from Department of Pulmonary Medicine, Sree Gokulam Medical College & Research foundation, were reviewed for a period of 2 years. Patient related information was collected from the Test Requisition Forms.

Each sputum samples received in the lab from the centers were divided; one part was immediately tested using GeneXpert, second part used for ZN smear microscopy on same day.

GeneXpert testing was performed according to the manufacturer's instructions.[13] Sample reagent was added to untreated sputum at a ratio of 2:1, manually agitated and kept for 10 min at room temperature, then shaken again and kept for 5 min; 2 ml of the inactivated material was transferred to the test cartridge and inserted into the test platform. Only electronic results were used for comparison. Direct smear microscopy was performed to investigate presence of acid fast bacilli in sputum samples using conventional ZN staining method. Slides showing red coloured acid fast bacilli were taken as positive and negative slides were those without any acid fast bacilli.[10]

Analysis

The data was tabulated in Microsoft excel sheet in a master chart and studied for correlation. Sensitivity, specificity, PPV(positive predictive value) and NPV(negative predictive value) was calculated.

The sensitivity, specificity, PPV and NPV for the diagnosis of Pulmonary tuberculosis was calculated for AFB smear microscopy and GeneXpert, using culture of *Mycobacterium tuberculosis* from sputum specimens as gold standard. By taking culture method as reference, samples that were positive and negative in culture were considered true positive and true negative. Culture negative and GeneXpert positive samples were taken as false positive samples. GeneXpert negative and culture positive samples were considered false negative.

RESULTS

Table 1: Demographic characters.

	Sputum positive	Sputum negative
Age in years(mean±SD)	48±15	45±20
Male female ratio	3 ; 1	2.6 ; 1
DM	14	10

Table 2: Overall Sputum smear positive versus GeneXpert positives.

	No cases examined	No of cases diagnosed	Diagnostic yield
Smear microscopy	98	21	21.42
GeneXpert	98	31	31.63
culture	98	32	32.65

Table 3: Sputum smear positive versus GeneXpert positives in all tested cases.

	Smear positive (21)	Smear negative (78)
GeneXpert positive(31)	21	10
GeneXpert negative(67)	0	67

Table 4: GeneXpert.

	sensitivity	specificity	negative likelihood ratio	disease prevalence	PPV	NPV	accuracy
value	96.88%	100%	0.03	32%	100%	98.55%	99%
95% CI	83.7-99.9%	94-100%	0-0.22	23-42%	90-99.7%		94.5-99.7%

Table 5: ZN staining.

	sensitivity	specificity	negative likelihood ratio	disease prevalence	PPV	NPV	accuracy
value	65.60%	100%	0.34	32.6	100%	85.70%	88.70%
95% CI	46.8-81.4%	94.5-100%	0.21-0.55			78.8-90.6%	80.8-94.2%

A total of 98 patients were included in this study, Mean age of the subjects was found to be 48±15 in diganosed TB patients with male predominence. Distribution of age, gender and Diabetic history of study subjects are shown in table 1.

Of the 98 specimens, 66 sputum samples were negative by all three methods used. 21sputum samples were ZN staining positive & 31 samples (31.6%) were GeneXpert.

TB positive. Out of 77 ZN staining negatives samples, 10 were positive in GeneXpert.

Among the 98 samples, 21 samples were ZN staining, culture and GeneXpert positive, 10 samples were GeneXpert positive and one sample was only culture positive. All these AFB smear positive samples were culture and GeneXpert positive [Table 3].

Overall sensitivity, specificity, PPV and NPV of GeneXpert when culture was taken as reference method is illustrated in [Table 4]. Overall sensitivity, specificity, PPV and NPV of AFB smear microscopy when culture was taken as reference method is shown in [Table 5].

DISCUSSION

In developing countries rapid and accurate diagnosis of tuberculosis is a challenge.[14] Confirmed laboratory diagnosis of tuberculosis is pivotal for management of disease and reduce the transmission of infection. Improved detection of tuberculosis is considered a priority by World health organization.[15] However the current frontline diagnostic test, smear microscopy, lacks sensitivity. A large number of cases remain undiagnosed by traditional sputum microscopy. Due to the slow growth of *Mycobacterium tuberculosis* and need for sophisticated lab facility, culture is available only in reference laboratories. Therefore, diagnostic delays in detection of smear negative pulmonary samples is of major concern. In the absence of alternative tests, such cases would remain undetected and unreported. The GeneXpert has been introduced with the aim to increase the detection of tuberculosis.[16,17]

In this retrospective study, we have evaluated the diagnostic yield of GeneXpert to detect MTB in Sputum samples. GeneXpert is a top point of care diagnostic assay that can be performed with minimal training. The results will be available within 2 hours, were the culture report will take weeks to come positive.[15,18]

Numbers of studies have demonstrated the utility of GeneXpert in diagnosis of pulmonary tuberculosis.[19-20] In our study, overall sensitivity, specificity, PPV and NPV of GeneXpert were 96.8%, 100%, 100%, & 98.5% respectively that is comparable with other studies.[21-24] In the other studies, GeneXpert sensitivity and specificity for sputum sample was from 81%-92% and 71%- 100%, it is in conjunction with our studies.[21,22,24-26] Although sensitivity in our study is 96%, it is because one sample MTB growth is in culture but it is possible that the bacterial load may have been too low for the GeneXpert to detect the DNA from MTB- complex. It shows that a patient with a negative GeneXpert can still have TB with MTB or MOTT.[21,25] Comparison to other studies, NPV value of GeneXpert is high in our study.[26] In our study, sensitivity and specificity of GeneXpert in sputum assay is 96% and 100% respectively, that is line with the study of Sharma et al., (96.9% and 99.8%).[24]

In comparison with culture used as gold standard, sensitivity, specificity, PPV and NPV for Smear microscopy for sputum sample were recorded as 65.6%, 100%, 100% and 85.7% respectively, which is in line with other studies.[21,25,26] Overall Specificities of GeneXpert and smear microscopy were 100% which also correlate with other studies.[21,25,26] Out of 78 ZN staining negative cases, MTB detected by GeneXpert in 10 samples, that correlate well with other studies. For smear positive cases sensitivity is 100% in line with other studies from 68.6%-100%.

As endorsed by WHO, our study further strengthens the use of GeneXpert in Tuberculosis suspected pulmonary samples. However, GeneXpert does not eliminate the need of conventional microscopy, culture and anti-tubercular drug sensitivity that are required to monitor the progression of treatment and to detect resistance to drugs other than Rifampicin.

Limitations

Number of samples in this study is less, further studies needs to be done with more number of samples. This study was performed retrospectively and results couldn't be correlated with radiological findings and histo-pathological reports. One of the important strength of the GeneXpert assay is its ability to detect the presence of Rifampicin resistance. The sensitivity and specificity of GeneXpert to detect Rifampicin resistance was not evaluated in our study

CONCLUSION

GeneXpert can be a useful diagnostic method in patients of suspected pulmonary tuberculosis either AFB smear negative or positive due to its rapidity. GeneXpert and AFB smear microscopy share almost same specificity but sensitivity of GeneXpert is much higher than AFB smear microscopy in respiratory samples. Although culture is

considered as a gold standard method but as it takes days to come positive and simultaneous detection of Rifampicin resistance is not possible with it. On other hand cost effectiveness of GeneXpert in low income countries with high prevalence of tuberculosis like India need to be done. The other major advantage of GeneXpert is that it simultaneously detects Rifampicin resistance and is beneficial in patient with MDR and HIV associated tuberculosis and should be studied further.

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