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Role of cartridge-based nucleic acid amplification test for early diagnosis of pulmonary tuberculosis

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Abstract
Introduction: The global burden TB remains enormous. Most of them occur in resource limited settings. Smear microscopy is the cornerstone but it has only modest sensitivity and poor positive predictive value. Culture is the gold standard for final determination but its results typically not available for 2-6 weeks. Given the inability to elucidate the drug resistance and rapid detection by these methods, preventing the patients from obtaining the appropriate and timely therapy at point of need.
Gene Xpert system offers an overall sensitivity that is higher than AFB smear microscopy, and also provides genetic information about mutations that confer antibiotic resistance to the primary antibiotic Rifampicin. Here, we describe an assessment of the Gene drive MTB/RIF assay using sputum samples and comparing its performance against standard AFB smear microscopy.
Objectives: 1. To assess the diagnostic usefulness of Gene-Xpert MTB/ RIF assay technique in management of tuberculosis.
2. To detect the prevalence of rifampicin resistance among smear positive and smear negative cases.
Material and Methods: This is an observational study conducted in the department of microbiology. Sputum sample of clinically suspected cases of pulmonary tuberculosis will be collected under all aseptic precaution and subjected to Gene Xpert for MTB/RIF detection after AFB microscopy.
Result: 33(22.29%) Sputum/BAL samples were AFB smear positive and 115(77.70%) % were negative. In Gene Xpert MTB/RIF assay 63(42.56%) were MTB positive and 85(57.43%) were MTB negative. Chi square test was applied; P value is <0.001. All results are highly significant. The Gene Xpert MTB/RIF Assay also detected RIF resistant 09(6.0%) and RIF susceptible 53(35.81%) cases and 01(0.67%) indeterminate case.
Conclusion: CBNAAT helps in increased case detection in lesser time to diagnose pulmonary tuberculosis as compared to conventional sputum microscopy. It also detects the rifampicin resistance with high specificity to start early treatment.

Keywords: CBNAAT, M. tuberculosis, AFB, MDR TB, microscopy

Introduction
Tuberculosis is one of the major global health problems and prevalence of TB is high among the developing world [1]. More than 9 million new M. Tuberculosis cases and 1.7 million deaths occur annually worldwide, mostly in resource limited settings [2]. Twenty five percent of global annual TB incidents occur in India making it highest Tuberculosis burden country [3].
Smear microscopy is the cornerstone for the diagnosis of TB in resource limited settings but it has only modest sensitivity (35-80%) [2]. Sputum smear microscopy is inefficient due to its variable sensitivity particularly in patient with sputum smear-negative and/or extrapulmonary diseases, and drug resistant TB [4-6]. Besides technical expertise and biosefety concerns, Lowenstein-Jensen (LJ) method, "the gold standard test" takes several weeks to produce results causing delayed onset of treatment. Multiple approaches to improve diagnosis of TB are in development. One test, Gene Xpert MTB/RIF, which was recently endorsed by the World Health Organization (WHO), has the potential to lead a revolution in the diagnosis of active TB disease and multidrug-resistant TB [2].
Gene Xpert MTB/RIF assay is a Cartridge-based fully automated nucleic acid amplification test currently recommended by WHO and adopted by Revised National Tuberculosis Control Programme run by Government of India for detection of tuberculosis case and rifampicin resistance [7]. The principle of Gene Xpert assay being detection of MTB and RIF resistance by polymerase chain reaction based amplification of rpoB gene segment and probing for mutation that are related to rifampicin resistance and it completes within 2hrs [7].

The aim of this study is to determine the diagnostic usefulness of the MTB/RIF assay for the diagnosis of tuberculosis and rifampicin resistance in pulmonary specimens.

Materials and Methods

This was the observational study conducted by Microbiology department of Dr. Panjabrao Deshmukh Memorial Medical College, Amravati from Jan 2017 to Dec 2017. This study was conducted after approval of institutional ethical committee. All patients attending the outpatient department and indoor patient department of various wards/units of tertiary care centre and referred cases from peripheral private hospitals were included in the study.

Inclusion criteria

Patient included in the study were of both sex & of any age, clinically suspected pulmonary tuberculosis, with or without abnormal chest radiograph compatible with pulmonary tuberculosis. Patients receiving anti-tuberculous drug were excluded from study.

Microbiological investigation

Sputum microscopy for acid fast bacilli-two sputum samples, one sample collected on spot under supervision and other collected early in the morning. All sputum sample obtained were decontaminated with N-Acetyl-L-cysteine with Na OH and processed in the standard manner by Ziehl and Neel sen staining. Smear grading was done according to RNTCP.

Sputum for CBNAAT-one sputum sample was collected in sterile container and was analysed by CBNAAT on Gene Xpert MTB/RIF manufactured by Cepheid, endorsed by WHO and run by revised national tuberculosis control programme as shown in fig no. 1 The Sample was diluted with sample reagent in 2:1 ratio, and then incubated at room temperature for 15 min. As shown in fig no. 2. With in between shaking and 2 ml diluted sample loaded into the cartridge for automated analysis with results in 2 hrs. The cartridge incorporates a syringe drive, a rotary drive and a filter upon which M. Tuberculosis bacilli are deposited after being liberated from clinical material. The test platform employs a sonic horn that insert into the cartridge base to cause ultrasonic lysis of bacilli and release of genetic material. The assay then amplifies a 192bp segment of the rpoB gene using hemi-nested rt-PCR. Mycobacterium tuberculosis is detected by the five overlapping molecular probe (probe A-E) that collectively are complimentary to the entire 81 bp rpoB core region [8-9]. M. Tuberculosis is identified when at least 2 of the 5 probes give positive signals. The result of sample processing control, allow the test to distinguish among the following results: M TB not detected, MTB detected Rif Resistance not detected, MTB detected Rif Resistance detected, MTB detected Rif Resistance indeterminate, invalid result or no result [4-10].

Fig 1: shows CBNAAT instrument

Fig 2: shows Cartridge with sample diluents

Statistical analysis

Data analysis was done using SPSS (Statistical package for social science) software SPSS (version 22.00). All results were analysed statistically by applying chi-square test and P value <0.05 was considered to be statistically significant.

Results

From Jan 2017 through Dec 2017, 148 sputum/Bal samples of suspected pulmonary tuberculosis patients were enrolled in the study. All samples were subjected to Ziehl and Neel sen staining and CBNAAT which included 89(60.1%) male and 59 (39.86%) female. Most of them, 32(21.62%) are between the age group of 26-35 yrs as shown in table no.1.

Table 1: Age and Sex wise distribution of Patients

Age groups (yrs.)	No. Of Patients		Total
	Male	Female	
13 months	01	00	01
14-25	12	14	26
26-35	21	11	32
36-45	15	11	26
46-55	14	07	21
56-65	17	11	28
66 & above	09	05	14
Total	89	59	148

Ziehl and Neelsen staining was done for 148 samples of cases who were having history suggestive of pulmonary tuberculosis. Out of these 33(22.29%) sputum/BAL samples were AFB smear positive and 115(77.70%) were negative. Then all the samples were tested on Gene Xpert MTB/RIF assay. Out of these 148 patients, 63(42.56%) were MTB detected and 85(57.43%) were MTB not detected. The MTB/RIF assay detect the agent in 32 out of 33 AFB smear positive cases and 31 out of 115 AFB smear negative cases as shown in table No. 2. So positivity of MTB detection by Gene Xpert 63cases (42.56%) is more as compared to ZN staining 33cases (22.29%). Out of 33 sputum positive cases, 32 were positive for MTB by Gene Xpert but in 01 sputum positive case MTB was not detected by Gene Xpert assay.

Table 2: Relation of MTB detection by Gene Xpert with AFB staining.

Gene Xpert (CBNAAT)	AFB + ve cases	AFB-ve cases	Total
MTB + ve	32	31	63
MTB-ve	01	84	85
Total	33	115	148

It is evident from the table no. 2 that Gene Xpert is highly effective than ZN staining for the diagnosis of Mycobacterium tuberculosis in pulmonary tuberculosis suspected cases. P value is highly significant (P<0.001). It also detects Rifampicin resistance to diagnose MDR TB. As shown in table no.3, 9(6.08%) cases were rifampicin resistance, 53(35.81%) cases were rifampicin sensitive & 01(0.67%) case was indeterminate out of 148 suspected cases. The overall prevalence of rifampicin resistance among suspected cases of pulmonary tuberculosis was 14.28% (9/63). Among the 9 rifampicin resistant cases, 5(7.93%) cases were sputum positive and 4(6.34%) cases were sputum negative as shown in table no.4.

Table 3: Relation of Rifampicin resistance with MTB positive cases.

RIF Resistance	MTB + ve	MTB-ve	Total
Not detected	54	85	139
Detected	09	0	09
Total	63	85	148

Table 4: Distribution of cases Rifampicin resistance (by sputum microscopy & Gene Xpert)

Sputum/BAL microscopy	MTB detected by Gene Xpert		
	Rif resistance not detected	Rif resistance detected	Total
Sputum Positive	27	05	32
Sputum Negative	27	04	31
Total	54	09	63

Discussion

Early diagnosis of TB is necessary to disrupt the disease transmission chain. Although ZN smear positive patients are considered highly infectious and being focused by most of the clinicians, smear negative are also reported to be responsible for approximately 17% of transmission and its impact on public health could not be neglected [11]. Therefore we evaluate the performance of Gene-Xpert assay in samples of suspected pulmonary tuberculosis with conventional microscopy.

In the present study most of the cases 32(21.67%) were in the age group of 26-35 yrs. and male to female ratio was 1.5:1 comparable to another study that showed mean age 30.1 years with male to female ratio 1.65:1 [12]. This shows that most of the MTB patients lie in the productive age 21-50yrs in the present study, so it is obligatory to increase the detection rate by using single or combined techniques which in turn can prevent greater economic loss.

ZN smear positivity in present study was found to be 22.29% and Gene Xpert positivity for MTB remained 42.56%. Rehman S, Iqbal R, etc from Lahore in 2013 showed 53% smear positivity by ZN staining and 82% MTB positivity by Gene Xpert assay [13]. However study done by Munir M, etc in 2015 from Lahore showed high smear positivity 67.5% as compared to 77.4% positivity MTB by Gene Xpert assay [11]. It has been stated that conventional laboratory method of ZN smear technique requires a bacillary load of 10^5/ml to show positivity, therefore making it an unreliable technique in the diagnosis of TB. However culture method considered as Gold-standard for detection and to know the drug sensitivity in TB but it is time consuming and requires strict biosefety infrastructure and trained laboratory staff.

In this study, out of 33 sputum positive cases 32 were positive for MTB by Gene Xpert assay and only one case was negative for MTB by Gene Xpert assay. The reason for false negative Xpert MTB/RIF assay result may be limited number of bacilli in this sample or there may be growth of MOTT [14]. Compared to the above techniques, Gene-Xpert assay has the advantages of less turnaround time, high sensitivity of detection of MTB with simultaneous assessment of Rifampicin resistance and has the potential to replace standard culture method. It has further been documented that Gene Xpert increases the detection rate by 10-15% compared to smear method in sputum samples [14].

In this study, we found the prevalence of rifampicin resistance among suspected cases of pulmonary tuberculosis without treatment was 14.28%. The reported prevalence of rifampicin resistance were 28.2% by Malhotra *et al*. [15] in 2002 from Jaipur, 33.7% from New Delhi by Jain *et al*. [16] in 1992 and 37.47% from Gujrat by Shah *et al*. [17] in 2002.

Conclusion

Gene Xpert assay detects MTB with more efficacy than sputum microscopy, also helping in early diagnosis in less than 2 hrs. It also detects rifampicin resistance with high specificity and can be used for screening for MDR-TB, so that early therapy can be started, thus decreasing the incidence of MDR-TB. And proved itself an effective tool in initiation of early treatment.

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