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DETECTION OF PULMONARY AND EXTRA-PULMONARY TUBERCULOSIS FROM CLINICAL SAMPLES WITH RIFAMPICIN (RIF) RESISTANCE BY GENE-XPERT MTB/RIF ASSAY AT A TERTIARY CARE TEACHING HOSPITAL, UDAIPUR, RAJASTHAN

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ABSTRACT Tuberculosis (TB) is one of the major concerns of health policy and rapid detection of M. tuberculosis and detection of rifampicin (RIF) resistance in infected patients are essential for disease management. Multi-drug resistance (MDR) TB is defined as tuberculosis (TB) disease caused by a strain of Mycobacterium tuberculosis (MTB) that was resistant to at least isoniazid and rifampicin (RIF). The aim of this study was to evaluate the importance of GeneXpert MTB/RIF for detection of Mycobacterium tuberculosis (MTB) with detection of rifampicin (RIF) resistance. In this study 967suspected cases of pulmonary and extra-pulmonary TB were included. Their sputum and other (Pus, CSF, tissue, etc.,) samples were collected and subjected to smear microscopy test followed by GeneXpert MTB/RIF assay. Of total 967 samples, 470 (48.60%) were positive for Mycobacteria by GeneXpert MTB/RIF and 118 (12.20%) samples were positive by ZN smear. Among 967 positive cases, 818 (84.59%) were pulmonary, 149 (15.41%) were extra-pulmonary (EP). Two ZN smear positive cases were negative by GeneXpertMTB/RIF. Of the 470 GeneXpert positive samples for MTB, 29(6.17%) cases showed RIF resistance and diagnosed as MDR-TB. GeneXpert MTB/RIF assay is efficient and reliable technique for the rapid diagnostic of TB. The GeneXpert MTB/RIF assay is a simple, high sensitivity and specificity for RIF resistance detection implies for diagnosis of MTB and RIF resistance in MDR cases as well as suspected smear negative and extra-pulmonary cases.

KEYWORDS : Mycobacterium tuberculosis (MTB), rifampicin (RIF) resistance, GeneXpert MTB/RIF, ZN stain

INTRODUCTION

Tuberculosis (TB) is the most common infectious disease worldwide caused by Mycobacterium tuberculosis (MTB) has elicit the need for rapid diagnostic techniques [1]. According to WHO the first milestones of the End TB Strategy, set for 2020, are a 35% reduction in the absolute number of TB deaths and a 20% reduction in the TB incidence rate, compared with levels in 2015. WHO worldwide report in 2015, there were an estimated 10.4 million incident TB cases. An estimated 62% of these cases were male, and 90% of cases were adults and 10% children. Six countries accounted for 60% of the global total: India, Indonesia, China, Nigeria, Pakistan and South Africa. The rate of progress in these countries will have a major influence on whether or not the 2020 global milestones are achieved.

India has the highest burden of both tuberculosis (TB) and multidrug-resistant (MDR) TB based on estimates reported in Global TB Report 2016.2Although the available data suggest that the TB epidemic may be on the decline, the absolute number of new cases is still the highest. India accounts for about 24% of the global prevalence, 23% of the global incident cases, and 21% of the global TB deaths.[3] Taking into consideration the magnitude of the disease burden, the Government of India has announced its plan to eliminate TB by 2025 during the Union Budget 2017–2018.

Indian Government launched National Tuberculosis Programme in 1962. However, the desired outcome could not be achieved. The program was reviewed, and then, Revised National TB Control Program (RNTCP) was launched in 1993 on a pilot basis. Yet by 1998, it covered only 2% of the population. By 2006, entire nation was covered by the RNTCP.[4] Although RNTCP has made great strides in the last decade, but it is still facing challenges for example microscopy is still the mainstay of diagnosis, the disease is distributed unevenly throughout the country which makes it difficult to achieve the goals.[5] Moreover, India has been in the news because of the international attention around the emergence of "totally drug-resistant" TB in Mumbai and the growing concern that routine TB control (i.e., the directly observed treatment, short-course strategy) may not be sufficient for reducing TB incidence in the country.[6]

Over the last National Strategic plan (NSP 2012–2017) period, significant gains were made. This includes mandatory notification of all TB cases, integration of the program with the general health services (National Health Mission), national drug resistance surveillance, and many more. However, more needs to be done to drastically reduce the TB incidence in India.

The best estimate is that there were 1.4 million TB deaths in 2015, and an additional 0.4 million deaths resulting from TB disease among HIV-positive people [7]. In terms of cases, the best estimates for 2015 are that there were 10.4 million new TB cases (including 1.2 million among HIV-positive people), of which 5.9 million were among men, 3.5 million among women and 1.0 million among children. Overall, 90% of cases were adults and 10% children. The only WHO-recommended rapid diagnostic test for detection of TB and rifampicin resistance currently available is the Xpert MTB/RIF® assay. The Xpert® MTB/RIF assay (Cepheid Inc., CA, USA) marks an important development in the field of rapid molecular TB diagnostics [8,9]. This multifunctional diagnostic platform is an automated, closed system that performs real-time PCR and can be used by operators with minimal technical expertise, enabling diagnosis of TB and simultaneous assessment of rifampicin resistance to be completed within 2 h. Sputum samples can be analyzed with very minimal processing, yielding positive diagnoses in 99–100% of patients with smearpositive pulmonary TB and 57–83% of patients with smear-negative pulmonary TB in clinical evaluation studies.[9] The only rapid test (Xpert® MTB/RIF assay) was initially recommended (in 2010) for diagnosis of pulmonary TB in adults. In October 2013, the WHO released a policy update on GeneXpert (MTB/RIF) which expands the recommended use of GeneXpert(MTB/RIF) as the initial diagnostic test in all individuals (smear negative, pediatric, MDR-TB and HIV) suspected of having pulmonary and extra-pulmonary tuberculosis (EPTB)[10,11].

MATERIALS AND METHODS

Clinical Samples:

In this study, pulmonary and extra-pulmonary samples obtained from different clinical departments were included. Confirmed sputum smear positive cases also included in this study for detecting the drug resistance.

Processing of samples AFB smears:

Before processing of specimens by Xpert MTB/RIF assay, smears were prepared and stained by the Ziehl-Neelsen (ZN) method and examined with a light microscope for the presence of AFB.

Xpert MTB/RIF Assay Sputum:

Sputum samples were processed directly from Xpert MTB/RIF test, according to manufacturer's protocol. Briefly, 2.0ml of GeneXpert MTB/RIF sample reagent was added to 1.0ml of sputum/other specimen in a sterile container using a sterile pipette and the container was manually agitated twice during a 20 minute incubation period at room temperature. Then 2 ml of the inactivated material was

transferred to the test cartridge by a sterile disposable pipette (provided with kits). Cartridges were loaded into the GeneXpert. The interpretation of data from MTB/RIF tests was software based and not user dependent [12].

Other than sputum samples:

Other than sputum samples were concentrated by cytocentrifugation at 3000g for 20 minutes and the deposit was processed as for sputum sample using, ZN staining, Xpert MTB/RIF assay.

RESULT

A total 967 presumptive samples processed for GeneXpert, 470 (48.60%) were positive for Mycobacteria by GeneXpert MTB/RIF. Out of 967 clinical samples 118 (12.20%) samples were positive by ZN smear. Among 967 positive cases, 818 (84.59%) were pulmonary, 149 (15.41%) were extra-pulmonary (EP). Among 470 positive patients, 303(64.46%) were males and 167 (35.54%) were females. Out of 303 males, 20 (6.60%) were children, 283 (93.40) were adults and among 167 females, 23(13.77%) were children, 144(86.23%) were adults. Among all, the highest detection rate of MTB was found by GeneXpert 470 (48.60%) followed by ZN smear 118 (12.20%). Two ZN smear positive cases were negative by GeneXpertMTB/RIF. Of the 470 GeneXpert positive samples for MTB, 29(6.17%) cases showed RIF resistance and diagnosed as MDR-TB.

Table 1 Detection of Mycobacterium tuberculosis by GeneXpert comparing with AFB smear examination

Test Name	Male		Female		Total (*n=967)	
	Children (0-18Yrs)	Adults (≥19Yrs)	Children (0-18Yrs)	Adults (≥19Yrs)		
AFB Smear Positive	2	78	3	35	118	967
AFB Smear Negative	14	506	19	310	849	
GeneXpert MTB Detected	20	283	23	144	470	967
GeneXpert MTB Not Detected	24	269	18	186	497	

DISCUSSION

The increasing incidence of MDR-TB in India and other developing countries is a serious threat to tuberculosis control. The major problem in treatment of TB is delayed diagnosis without drug sensitivity which often leads to MDR-TB. World-wide prevalence of MDR-TB is markedly increasing which demands for the accurate and rapid method for the diagnosis. This will help clinicians in effective treatment, management and control of TB. Therefore the present study was designed to evaluate the importance of the new PCR-based technology (GeneXpert MTB/RIF) for the detection of MTB and RIF resistance.

According to the World Health Organization (WHO) 650,000 people are infected worldwide and 12 million suffer from TB. In Africa, 1.9% of new cases and 9.4% of diagnosed and treated patients are infected by MDR strain [13]. The results of our study (6.17%) showed that 29 strains harboring mutations in rpoB were phenotypically MDR-TB strains (resistant to RIF). This was very less comparable to 77.4% reported by Olusoji*et al.*[14]. Few studies had documented the presence of cases infected by MDR strains in Nigeria, with prevalent rates ranging from 4–76.3% [15,16], but was much superior to the results found by Rasaki*et al.* [17] where forty four (31.4%) were positive and to another rates published in previous studies from India [18,19].

There was male preponderance, 303 (64.46%) as against 167 (35.54%) female; this was in concord with the work of Ganguly *et al*. [20] where male subjects had prevalence of 85.71% as against 14.29% of females. Similarly, a European study by Faustini *et al*. [21] observed more TB cases among men. In a another study done by Robert *et al*. [22] the age and the sex distribution was similar to the study of Ganguly et al.[20] This disparity could be due to the fact that male subjects were more exposed to risk factors of TB infection. In the present study, the distribution according to age showed that the majority of patients with TB cases belonged to age group of ≥19 years followed by ≤18 years. This was in concord with the study of Thomas et al. [23].

In the current study the efficacy of the GeneXpert (MTB/RIF) for the diagnosis of TB was 470 (48.60%) from 967 clinical suspected cases of TB. In the present study GeneXpert for MTB GeneXpert showed 100% sensitivity and superior than ZN smear for detecting MTB. Two smear positive cases showed negative by GeneXpert. This is may be due to Mycobacterium other than tuberculosis (MOTT). Different studies about the performance of GeneXpert (MTB/ RIF) have showed the test sensitivities in the range of 57%to 76.9% in cases of culture positive/smear negative cases, while 98% to 100% sensitivities were observed in culture positive/smear positive cases. The overall specificity of GeneXpert remained at 99% to 100% [24-27]. Our finding that the Z-N smear is less sensitive than the GeneXpert MTB/RIF test is reasonable because the Z-N smear method requires 5x103 to 1x104 bacilli/ml of specimen to generate a positive result. However, the GeneXpert assay only requires 131 bacilli/ml [28, 29]. Additionally, the GeneXpert MTB/RIF assay detects DNA of *Mycobacterium tuberculosis* (MTB). In this study, two isolates were detected by ZN stain, but were negative with the Gene Xpert TB/RIF assay. This is because GeneXpert TB/RIF is a nucleic acid amplification test for detection of distinctive DNA of the MTB complex, exclusive of MOTT [13].

Multidrug-resistant tuberculosis (MDR-TB) is defined as TB caused by strains of M. tuberculosis that are resistant to at least isoniazid and RIF [30]. Mono-resistance to RIF is rare; however, 90% of RIF resistant isolates also exhibit resistance to isoniazid. Therefore, the detection of RIF resistance may serve as a surrogate marker for MDR M. tuberculosis[31]. For RIF resistance detection, Xpert® MTB/RIF provides accurate results and can allow rapid initiation of MDR-TB treatment[32]. In our study, 29 (6.17%) were RIF resistant, while 441 (93.82%) were RIF sensitive. Our study similar to the study of Olusoji et al. [14], Lawson et al. [34], Ganguly et al. [20], where (7.2%), (8.6%), (19%), isolates were resistance to RIF respectively and Idigbe et al. [35] who reported only 2% of resistance to RIF in Lagos, Nigeria. However, no strain of RIF resistant was reported in the findings of Rasakiet al. [17]. In study conducted by Guenaoui K et al. [36]. Trivedi [18] and Shah [37] reported 21 (42%) were RIF resistant, while 29 (58%) were RIF sensitive respectively. but lower to the study of Chowgule[32] who reported a very high incidence of RIF resistance of (66.8%)[38].

CONCLUSIONS

The Gene Xpert MTB/RIF assay is a simple, rapid, and accurate test method for detecting M.TB in sputum specimens, is less dependent on the operator's skills, and staff with minimal training can use the equipment. Although the Gene Xpert MTB/RIF assay has these advantages, similar to other tests for M. TB, a negative result cannot exclude the diagnosis of TB, and patients with positive results can also be assessed comprehensively with results of the Z-N smear test, culture, clinical symptoms, and radiographic evidence.

The high sensitivity and specificity of Xpert MTB/RIF for RIF resistance detection support its use as an initial diagnostic test for RIF resistance. Therefore, implementation of molecular approaches for direct diagnosis of MDR TB, as a part of routine analysis in the laboratories of health care institutions, would be of great benefit in adapting treatment regimens, limiting dissemination of MDR TB strains. A highly sensitive, cross-platform, diagnostic screening assay for the detection of *Mtb,* directly from decontaminated sputum was developed without a time-consuming nucleic acid extraction procedure making it more suitable for adaptation to point of care (POC) use and with a turnaround time of around two hour.

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