Role of Real Time PCR in Detection of MTB, MDR and XDR TB Directly from Sputum Specimens of Clinical Suspects of PTB

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Abstract

Background and Objectives: “Presently, Sikkim has emerged as the next big concern on India’s already riddled tuberculosis map with 11 per cent of the new tuberculosis cases in the State found to be multi-drug-resistant”; as reported in The Hindu; 14th July 2016. The study intended in verifying the role of real time PCR in the detection of Mycobacterium tuberculosis, diagnosis of multi drug resistant tuberculosis and extensively drug resistant tuberculosis directly in sputum samples of pulmonary tuberculosis.

Materials and Methods: The sputum samples were subjected to fluorescence staining and real time PCR. For the detection of MDR and XDR TB cases the genes targeted were rpoB- rifampicin resistance, katG and inhA- isoniazid resistance, gyrA-fluoroquinolone resistance, rrs, eia-10 and eia-37- resistance to injectable drugs.

Results: The study revealed 50(61%) sputum positive and 32 (39%) sputum negative cases. However, real time PCR diagnosed 64(78%) positive cases. Fourteen MDR TB and 1 XDR TB cases were diagnosed simultaneously, with maximum MDR TB cases belonging to 3+ AFB grading.

Interpretation and Conclusion: Real time PCR showed better sensitivity, specificity and an early means of detection of Mycobacterium tuberculosis as well as multi drug resistant tuberculosis and extensively drug resistant tuberculosis in the suspected samples.

Keywords: Real time PCR, Multi drug-resistant tuberculosis, Extensively drug-resistant tuberculosis, Fluorescence staining.

Introduction

India is one of the highest tuberculosis (TB) burden countries accounting for nearly one-fifth of the global incidences with an estimated incidence of 1.9 million cases from India. Furthermore, about 500,000 new multi drugs resistance tuberculosis (MDR TB) cases are estimated to occur every year.¹ MDR and extensively drug resistant tuberculosis (XDR TB) poses a great threat to the TB control programmes worldwide. Early diagnosis of drug resistance TB among the suspected patients can allow to quickly start the appropriate lifesaving treatment and also curb the transmission of the infection and disease.
It is important to provide prompt diagnosis and appropriate treatment. Real-time PCR thus can provide results for better and early treatment of pulmonary tuberculosis (PTB) as well as MDR and XDR TB. The emergence and spread of MDR and XDR TB are mostly due to the non-compliance and incomplete treatment. Today, treatment for drug-resistant TB can take up to two years, and is so complex, expensive, and toxic that many patients are unable to access treatment. Of those who do, more than a third will die.4 Once a drug-resistant strain has developed, it can be transmitted directly to others just like drug-susceptible TB.5 Today, it is believed that direct transmission is the most common way drug-resistant TB is spread, and augments this emerging global health threat.6 Therefore, diagnosing drug resistant TB among the patients in the initial stage can allow the clinicians to start the appropriate treatment quickly.

Materials and Methods

Study setting and study population
A cross-sectional study was conducted in the Department of Microbiology, Sikkim Manipal Institute of Medical Sciences (SMIMS) and Central Referral Hospital (CRH), Tadong, Sikkim from December 2016 to July 2017. Institutional Ethical clearance was obtained prior to the study. The sample size calculation was done by the standard sample size calculation method. First 82 new clinical suspects of PTB, not on anti-drug regimen and provided consent were included in the study.

Sputum Smear Microscopy
Two sputum samples: one spot and one early were collected in a sterile, leak proof 5-10ml container and were transported to the microbiology Tuberculosis laboratory. Auramine-O staining was performed to identify sputum positive and sputum negative samples.

Specimen processing for real time PCR
All the collected sputum samples were processed by N-acetyl L-cysteine- sodium hydroxide (NALC-NaOH) method.7 After decontamination the samples were re-suspended in sterile phosphate buffer solution (pH 6.8). Extraction was carried out using HELINI™ PureFast Bacterial Genomic DNA Minispin prep Kit (HELINI Bio molecules, Chennai) (Cat. No. 200218-100 purifications) as per the user guidelines. The eluted purified DNA was then stored at -20°C with the volume adjusted to 100μl. Detection of MTB, MDR and XDR TB by real time PCR.

A detection was carried out as per the instructions provided in the user’s manual.

Interpretation of the result
Internal control CT value was expected between 24 to 31. However, as stated in the user guidelines CT values of 31±3 were also interpreted within the normal range, considering the extraction efficiency, the quality of elite added to the PCR reaction and the individuals machine settings.

Result and Discussion
Delay or missing the cases of TB by conventional methods has drawn attention to use molecular methods for diagnosis of the cases along with the mutation in genes responsible for drug resistance. With the uncontrolled spreading of TB in the society, it is important that TB along with MDR and XDR TB is diagnosed early and efficiently. Amongst the total study population of our study50 (61%) cases out of 82 sputum samples were smear positive and 32(39%) were smear negative. Real time PCR detected 64(78%) positive cases. Sputum smear microscopy is the defined best tool for the primary diagnosis of TB and also helps in monitoring the response of the patient to the medication but tends to miss or interpret a false negative result. Although patients with sputum smear-negative TB are less infectious, they are more likely to die during or before diagnosis than patients with smear positive TB, and contributes to TB transmission.8,9 Studies have shown the sensitivity (87%-100%) and specificity (98%-100%) of real time PCR in the detection of MTB.10,11,12
Of the total real time PCR positive cases 14 (22%) were MDR TB and 1(2%) case was XDR TB. Out of 14 MDR TB cases 1(7%) was mono resistant 13(93%) showed point mutations in genes responsible for drug resistance to one or more drugs, 2(14%) cases showed resistance to both katG and inhA for isoniazid and 1(7%) case showed resistance only to katG gene. All the 14 cases were resistant to rpoB gene for rifampicin resistance. Only one case was found to be resistant to XDR TB and was resistant to gyrA and eia-10 genes. The diagnosed MDR and XDR TB were found to be present only amongst the 50 sputum smear positive cases with the dominance of MDR and XDR TB in +3 AFB graded patients. Real time PCR was more efficient in detection of MTB cases and could detect 14(17%) additional cases of MTB that were sputum negative.

The major advantage of our study was the detection of MDR and XDR TB directly from the sputum sample along with the determination of the specific gene causing resistance in the individual at focus. Related studies have provided an insight equivalent to our study with a few similar targeted genes in reference to sensitivity, specificity, reliability of the technique in detection of pulmonary tuberculosis and MDR status directly from the sputum sample. Studies have interpreted the results as 98% sensitivity and 100% specificity for rifampicin resistance, 85% and 100% for isoniazid while using a molecular beacon for rapid drug susceptibility testing. It was also reported that real time PCR has a sensitivity of 85% and 98% for the detection of MDR TB strains. The result for the determination of clinical efficacy of the real time PCR in the detection of MDR TB was found to be 100% concordant with Gene Xpert. The present study also aimed at the diagnosis of XDR TB among the smear positive as well as smear negative cases, with the diagnosis of one XDR TB case. Traditional TB culture and drug susceptibility testing takes 6 weeks or more to provide a reliable result. However, in our study the total time for process the result was two days adding to our advantage. Thus, providing a scope for the efficient diagnosis of MDR and XDR TB accurately, directly and in a lesser span of time, to provide the best possible treatment to the infected individuals with the accurate treatment without a doubt or delay. Finally contributing to better management of patients as early as the initiation of the treatment.

There were some limitations; first, the small number of suspected TB patients were present as it is a central referral hospital and patients included were only the ones who attended CRH for their treatment. Therefore, the clinical findings of our study may also vary depending on the load of the disease in this region. Second, the drug susceptibility testing was not performed for second line drugs; thus, unable in interpret the result. Nevertheless, our results are consistent with the previous studies in detecting MTB and resistance to rifampicin.

Conclusion
Real time PCR helped in the detection of MTB cases amongst the smear negative cases due to its higher sensitivity and specificity; thus, providing an early and reliable result. However, it also detects MDR and XDR TB cases amongst the suspected individuals along with the responsible genes for mutation helping in the initial and early management of MDR and XDR TB cases before starting the DOTS therapy also limiting the wastage of time and side-effects of drugs therapy that a patient has to undergo before the detection of MDR and XDR TB.

References


