International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 10; Issue 08 (C); August 2021; Page No.25031-25034
DOI: http://dx.doi.org/10.24327/ijcar.2021.24890.4993

Research Article

TRUENAT- RAPID DIAGNOSIS OF MYCOBACTERIUM TUBERCULOSISAND RIFAMPICIN RESISTANCE IN PULMONARY SAMPLES IN TERTIARY CARE AND TEACHING HOSPITAL, DODA, J&K

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A R T I C L E I N F O

Article History:
Received 6th May, 2021
Received in revised form 15th June, 2021
Accepted 12th July, 2021
Published online 28th August, 2021

Key words:

Chip based assay, Rifampicin resistance, Tuberculosis, Truenat chip based micro PCR system, Truelab™ Real Time micro PCR Analyser.

A B S T R A C T

Tuberculosis (TB) is an infectious disease caused predominantly the bacillus Mycobacterium tuberculosis. It typically affects the lungs (Pulmonary TB) but can affect other sites as well (extrapulmonary TB). Tuberculosis (TB) is the second largest killer worldwide, after HIV and is the leading cause of death in HIV patients. Tuberculosis is major public health problem and second largest cause of death among infections disease. Pulmonary TB spreads through air and is highly contagious. Over 80% of TB infections are pulmonary and if left untreated, pulmonary TB patient can infect up to 10-15 other people through close contact over the course of a year. Molecular technique using Real time PCR technology has gained momentum for rapid diagnosis and detection of drugresistance. This was a prospective study done during the period from July 2020 to December 2020 to study the prevalence of Pulmonary Tuberculosis and Rifampicin Resistance in Doda district of Jammu and Kashmir by using Truenat chip based micro PCR system and to understand the demographics of the cases attending the tertiary care hospital. All the presumptive TB cases with chest symptoms attending Govt. Medical Hospital, Doda were included in the study. The procedure included three steps: first- sputum sample were subjected to routine Ziehl- Neelsen staining and examined under microscope carefully. Then all sputum samples were further processed by Truenat, a chip based nucleic acid amplification test (Molbiodiagnostics pvt. Itd,) that includes two steps: first sputum samples were processed for separation of DNA by using True prep device. Second –Presence of MTB was detected by Truelab™Real Time micro PCR Analyser. All the MTB positive samples were tested for presence of Rifampicin resistance by using another specialized chip. Atotal of 727 sputum samples were processed, out of which 47(6.16%) were positive for MTB. Rifampicin resistance was detected in 4 (8.51%). Positivity was more in males 65.95% as compared to females 34.42%. Truenat is a simple method and laboratory technician with minimal training can perform the procedure.

INTRODUCTION

Tuberculosis causes the highest number of deaths globally, despite the availability of potent anti-TB drugs. The global burden of TB remains enormous. As per Global TB report 2017, more than 9 million new tuberculosis cases and 1.6 million deaths occur annually worldwide. Estimated MDR/RR cases are 6 lakhs. India is the highest TB burden country. 1/4th of the global annual new cases occur in India. Incidence is 2.7 million cases annually and mortality is 4.35 lakhs per year (1). Over 25% of patients seeking care in India's public sector are neither diagnosed nor started on treatment (2). Tuberculosis (TB) is the second largest killer worldwide, after HIV and is the leading cause of death in HIV patients. Pulmonary TB spreads through aerosols and is highly contagious. Over 80% of TB infections are pulmonary and if left untreated, a pulmonary TB patient can infect up to 10-15 other people through close contact over the course of a year(3). Due to the highly infectious nature of pulmonary TB, it is important to diagnose and treat the disease very early. Despite the availability of highly effective treatment for decades, TB remains a major global health problem mainly because of poor case detection(4). The most common method for diagnosing pulmonary TB worldwide is sputum smear microscopy. However, sensitivity of direct smear microscopy is low and estimates range from 30% to 70%. It is even lower in case of HIV-infected patients. Culture is more sensitive than microscopy and is considered the current gold standard. Culture requires specialized and controlled laboratory facility and highly skilled manpower and takes 2 to 6 weeks to provide the results. Automated methods and Molecular techniques such

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as polymerase chain reaction (PCR) or Real Time PCR are much more sensitive than microscopy and culture.

However, these tests have so far been restricted to centralized reference laboratories as they require skilled manpower and elaborate infrastructure. Also, the turnaround time for results could take a few day (5,6). Moreover, Drug resistance is a major issue in the treatment of tuberculosis. Drug resistance is because of either mismanagement of TB patients- wrong diagnosis, delay in diagnosis, wrong or interrupted treatment and injudicious use of both first and second line drugs. Multiple approaches to improve diagnosis of TB are in development. Amongst these are CBNAAT (Gene X pert) and LPA, endorsed by WHO to be used in RNTCP for rapid diagnosis of MTB and detection of Rifampicin resistance (7). These require uninterrupted power supply, air conditioning system, proper infrastructure and trained personnel. Patients have to travel to nearest testing centre or samples need to be transported. This limits its use in peripheral settings and Active Case Finding program (8).

The need is for accurate, feasible, rapid, affordable, and if possible, near-point-of-case TB diagnostic tests for use in resource limited settings (9,10). The Truelab™ Real Time micro PCR System enables decentralization and near patient diagnosis of MTB by making real time PCR technology rapid, simple, robust and user friendly and offering "sample to result" capability even at resource limited settings. It is supplied by Molbio diagnostics pvt. ltd., Goa, funded by Bictec labs, India. This test has been evaluated by the premiere institutes for its sensitivity and specificity and has been recommended in health care settings (9,11). It is extensively validated and licensed by Indian FDA. It has sensitivity of 99% and specificity of 100%. The MTB strain H37Rv from Zeptometrix was used for LOD (Limit of Detection) determination. LOD was determined to be 100 CFU/ml in sputum sample.

This was a prospective study done during the period from July 2020 to December 2020 to study the prevalence of Pulmonary Tuberculosis and Rifampicin Resistance in Doda district of Jammu and Kashmir by using Truenat chip based micro PCR system and to understand the demographics of the cases attending the tertiary care hospital.

MATERIAL AND METHODS

Sputum samples of all the Presumptive pulmonary TB cases were collected in universal container. The sample was first subjected to routine Ziehl- Neelsen staining, then examined under oil-immersion of microscopecarefully. The smear grading was performed using the Revised National Tuberculosis Elimination Program(NTEP) guidelines. A specimen positive even for single AFB/100 fields was taken as positive for *Mycobacterium tuberculosis* and a minimum of two sputum samples (spot and morning) negative for AFB was evaluated for 100 fields was declared as negative.

DNA Extraction

2-5ml sputum sample was collected in sputum cup and labelled with patient details (Name, Age, Sex and Lab Id). Two drops of liquefication buffer was added to the sputum and cap was closed. Then the sputum cup was gently swirled to mix and kept to incubate for 10 minutes at room temperature. Then the sample was pipetted after 10 minutes, incubated for another 5 minutes with swirling at 2 minute interval. Then 0.5ml of this liquefied sputum sample was transferred in lysis buffer bottle with the help of 1ml transfer pipette. Then 2 drops of liquefication buffer was added into the lysis buffer swirled gently to mix and incubated for 3-5 minutes. Then cartridge was removed from pouch, labelled and placed on the cartridge stand (Figure 1). The elute collection tube (ECT) was taken and labelled and keptaside for later use. Then the entire content of the lysis buffer tube was transferred to sample chamber of cartridge using 3ml transfer pipette. Cartridge was then placedin the Auto V2 device and then start button was pressed to continue the extraction process (Figure 2) and device was allowed to beep at the end of the DNA extraction process and the cartridge holder was ejected automatically, cartridge was removed gently by pulling out the cartridge holder and then placed in the cartridge stand. Finallythe elute chamber was carefully pierced with the help of elute transfer pipette and the elute was transferred into elute collection tube. The transfer pipette and cartridge were finally discarded in 1% hypocholorite solution. The time taken for entire process was 20 mints.

To continue the process Truenat™ MTB chip (Figure 4) was placed on the chip tray of the Truelab™ Real Time micro PCR Analyzer (Figure 3). Six µL of the purified DNA was then dispensed with the help of micropipette provided and dropped into the microtube containing freeze dried PCR reagents and allowed to stand for 30 seconds to get a clear solution. Six µL of this clear solution was then pipetted out and dispensed into the reaction well of the Truenat™ MTB chip and the test was started by pressing the start button. The Cycle threshold (Ct) is defined as the number of amplification cycles required for the fluorescent signal to cross the threshold. A positive amplification causes the dual labelled fluorescent probe to release the fluorophore in an exponential manner which is then captured by the built-in opto-electronic sensor and displayed as amplification curve on the analyzer screen, on a real time basis during the test run. In the case of negative samples, amplification does not occur and a horizontal amplification curve is displayed on the screen during the test run.

At the end of the test run, a MTB "DETECTED" or "NOT DETECTED" result was displayed and in positive cases, quantitative value was also displayed on the screen. Based on the Ct of the internal positive control (IPC), the validity of the test run was also displayed. (The IPC is a full process control that undergoes all the processes the specimen undergoes - from extraction to amplification thereby validating the test run from sample to result) Figure 1.

The target sequence for this kit is part of the (*nrd*B) ribonucleoside-diphosphate reductase gene, the product of which provides the precursor for DNA synthesis. The region selected is specific to the MTB complex. The used Truenat™ MTB micro PCR chip was discarded in freshly prepared 0.5% sodium hypochlorite solution for 30 minutes before disposal as per the standard medical waste disposal guidelines. The samples which were positive for MTB were loaded into another chip designed for detection of rifampicin resistance (Truenat™ MTB/RIF micro PCR chip) and loaded into Truelab™ Real Time micro PCR Analyzer.

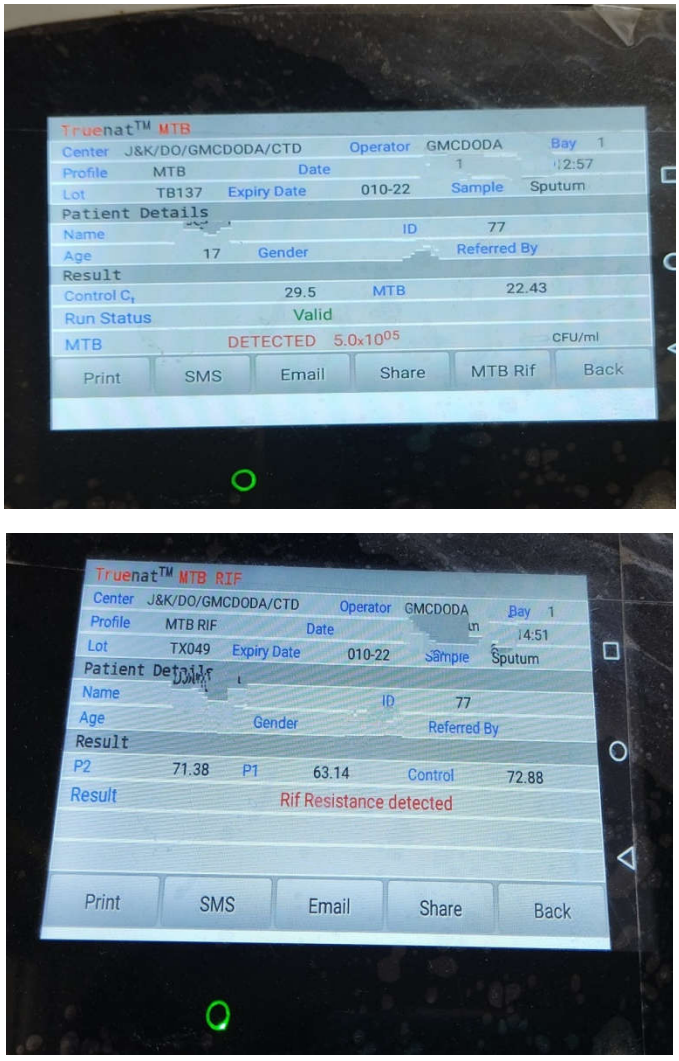


Fig 1

Fig 1 Trueprep®v2 AUTO Universal Cartridge Based Sample Prep kit

Fig 2 Trueprep AUTO Universal Cartridge Based Sample Prep device

Fig 3 Truelab® Duo Real Time Quantitative micro PCR Analyzer

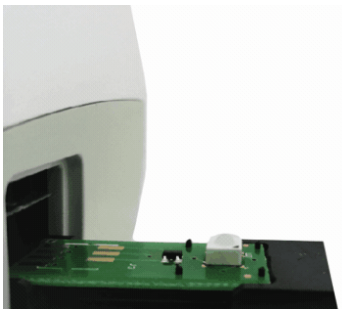


Fig 4 Truenat™ MTB Chip

RESULTS AND DISCUSSION

A total of 727 sputum samples were processed during the period from July 2020 to Dec 2020. Out of 727 sputum samples 47 (6.16%) were positive for MTB and 680(93.53%) were negative for MTB.

Out of 47 MTB cases, Rifampicin resistance was detected in 4(8.51%) and and 43 (91.48%) cases were sensitive to Rifampicin. Most of the sample were from age group 21-40(27.23%) followed by 10-20(15.68%). Out of 727 cases, Males were 513 (70.56%); Amongst them, MTB was detected in 31 (65.95%) and Rifampicn resistance seen in 3 (1.41%). Females contributed 214(29.43%) among them MTB was detected in 16 (34.42%) and Rifampicin resistance in 1(0.47%).

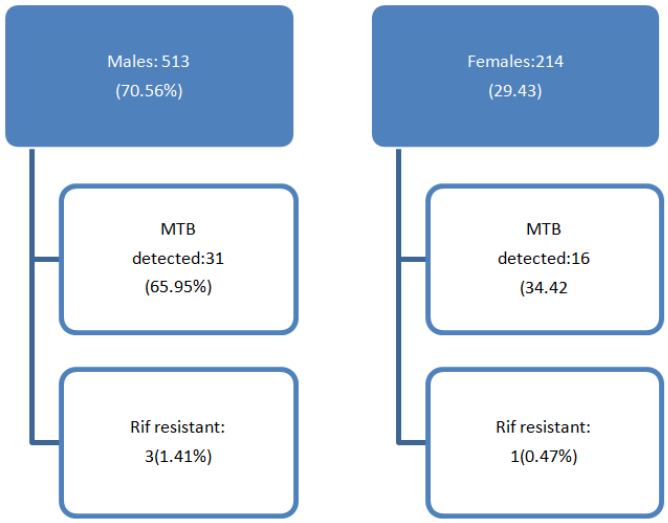
A major WHO priority for TB diagnostics is to implement a rapid, sputum based molecular test to replace smear microscopy at peripheral level. NTEP has recently developed National Strategic Plan (NPS) to work towards achieving the goal of eliminating TB by 2025. Drug resistant TB (DRTB) is a major impediment to achieve this goal. As per the Drug resistance survey conducted of MDR-TB in India in 2014-16, the prevalence of MDR-TB was 2.84% and 11.6% among new and previously treated TB cases respectively. Best method to control TB is early diagnosis and initiation of appropriate treatment. True Nat Real time quantitative micro PCR assay serves the need of the day as it is a rapid, portable and can be easily used in limited resource setting. The turn around time is

about one hour which allows same day diagnosis, same day treatment decision. A limitation of TruNat is that it cannot detect MDRTB to second line injectables which is of more significance in high burden countries like India. But as compared to other molecular method it is very effective. In the present study, out of 727 sputum samples, MTB was detected in 47 samples (6.16%). Rifampicin resistance was seen in 4 (8.51%). People in the age group of 21-40 were in maximum numbers (27.23%). Males were predominantly affected (70.56%). Rifampicin resistance was more in males (1.41%). (Table 1)

Table 1 Age wise distribution of samples

Age in year	No. of Samples
0-10	15 (2.06%)
11-20	114(15.68%)
21-30	198(27.23%)
31-40	96(13.20%)
41-50	67 (9.21%)
51-60	81(11.14%)
61-70	79(10.86%)
71-80	51(7.01%)
81-90	21(2.88%)
91-100	05(0.68%)
Total	727

RESULTS OBTAINED GENDER WISE



The performance of Truenat has been evaluated extensively by various researchers and has been compared with conventional culture based as well as with other molecular diagnostic methods. Nikam *et al.,* from Hinduja Hospital and Medical research centre, Mumbai evaluated the performance of Truenat RTPCR in comparison with GeneXpert on sputum samples from Pulmonary TB cases and found a high concordance(96%) with GeneXpert(9). In another study by Nikam *et al.,* Truenat MTB test was found to have sensitivity of 91.1% and in- house nested PCR a sensitivity of 90.5%(11). Though culture based methods are considered gold standard in the diagnosis of tuberculosis, molecular diagnostic methods have been used extensively in the diagnosis of tuberculosis because of the rapidity of the detection of cases which helps in early diagnosis and initiation of treatment. GeneXpert developed by Cepheid company, has long been considered one of the best molecular method because of the ease of doing as it is a cartridge based method which involves simple procedure. But the cost of the instrument and cost per test has been a major drawback for its extensive utilisation in peripheral setups.

Truenat PCR system is a cartridge based test for the rapid diagnosis of tuberculosis along with the detection of rifampicin resistance which is developed in India. With the extensive availability of this economically viable diagnostic method in resource poor country like India will not only help in the early diagnosis but also in the appropriate management and infection control measures and controls the spread of this deadly disease. Truenat will improve linkage-to-care and increase life expectancy also. Truenat MTB test is extremely useful test in resource limited health care settings such as designated microscopy centers (DMCs) and primary healthcare facilities in India. It is a rapid test, allows detection of TB in approximately one hour and can be utilized in near-care settings to provide quick and accurate diagnosis. As a portable platform it could also be utilized in ACF (Active case finding) programs, which are currently the need of the hour.

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