European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 10, Issue 2, Winter 2023

ORIGINAL RESEARCH

Molecular Diagnosis of Drug Resistant Tuberculosis by TRUENAT at a Tertiary Care Hospital

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Received: 15 January, 2023 Accepted: 28January, 2023

ABSTRACT

Background: Tuberculosis is most common infectious disease in developing countries. New rapid molecular diagnostics could dramatically increase TB detection and linkage-to-care, which are key components of both the World Health Organization's (WHO) End TB Strategy and India's National Strategic Plan for Tuberculosis Elimination 2017–2025.

Aim: To compare the Truenat with sputum smear microscopy.

Material and Methods: The study was conducted in a tertiary care hospital, Gujarat. 150 patients having symptoms related to tuberculosis were tested for sputum smear microscopy as well as by Truenat for molecular diagnosis of tuberculosis and rifampicin resistance at Microbiology laboratory.

Results: Out of 150 samples 54 samples shows positive results for Mycobacterium tuberculosis by smear microscopy and 69 samples shows positive results by truenat for detection of Mycobacterium tuberculosis. Amongst 69 samples 7 samples shows rifampicin resistance while rest 62 shows rifampicin sensitive.

Conclusion: As truenat MTB is portable, TrueNAT could increase treatment initiation by reducing turnaround time for test results and decreasing the need for laboratory referrals at developing countries.

Key words: Infectious Disease, Microscopy, Resistance, Truenat MTB

INTRODUCTION

Tuberculosis is the second largest killer in the world, after HIV and the leading cause of death. Pulmonary tuberculosis spreads through air & it is highly contagious. Over 80% of tuberculosis infections are pulmonary tuberculosis and if left untreated patients can infect up to 10-15 people through close contact over the course of a year.[1] However, estimated 10 million new tuberculosis cases in 2019, 2.9 million cases went undiagnosed. Only 61% of bacteriologically confirmed tuberculosis cases were tested for multi drug resistant, rifampicin (RIF) resistance. Conventional culture and drug susceptibility testing (DST) methods rely on

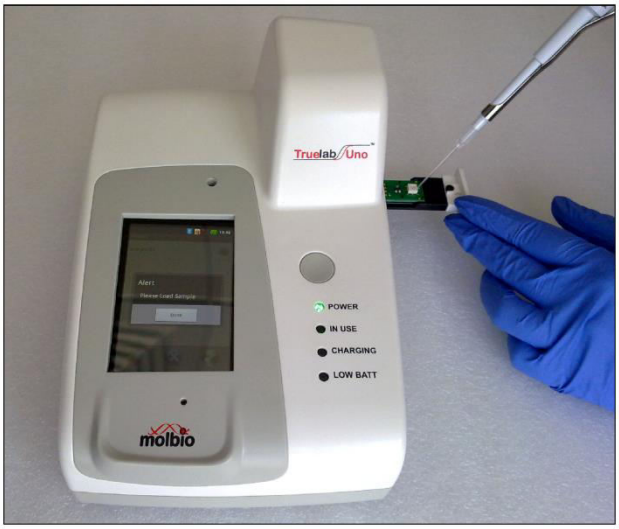
the slow growth of Mycobacterium tuberculosis in solid or liquid media, which can take 6-8 weeks to months to yield results and can lead to prolonged periods of ineffective therapy and ongoing disease transmission to community. Furthermore, many developing countries with high tuberculosis burdens lack the resources to establish the stringent laboratory conditions needed for these growth-based methods and must rely upon sputum smear microscopy tests (ZN stain and fluorescent microscopy) which, on average, detect only 45% of TB infections.[2] There has been ample interest in developing cost effective molecular tests that can be used ‘‘near-patient’’ as a means to curb the tuberculosis menace. With combined advantages of affordability, ease of use, diagnostic sensitivity and portability, low-cost, point-of-care molecular devices that enhance the efforts to treat diseases before they spread and cause irreparable damage to the patient’s health are good candidates for wide-scale use among the peripheral laboratories in India and other countries of South-East Asia, which accounts for 50% of the global burden of tuberculosis. It was reported that a novel tuberculosis test, the TrueNAT MTB, was able to detect tuberculosis rapidly with good sensitivity in comparison with a Composite Reference Standard (CRS). The test, TrueNAT, offered faster and accurate results.[3]

MATERIAL AND METHODS

This was a prospective cross-sectional study which was done in a tertiary care centre in Gujarat. Total 150 samples were received by microbiology laboratory during the period of December 2021 to January 2022 at a tertiary care hospital**.** The study population comprised adult men and women presenting to OPD with symptoms suggestive of pulmonary tuberculosis disease like cough of at least 2 weeks and any one or more of fever, night sweats, and/or weight loss.

All participants were instructed on how to collect sputum samples with a volume of at least 4 mL. Participants were asked to collect 2 sputum sample into 50-mL tubes until this volume was reached; a first morning specimen and a subsequent second specimen was collected on the spot at OPD or as a second morning specimen as possible. Specimens were stored at 2 to 8°C and transported to the Microbiology laboratory, where all laboratory testing was performed.[4]

Figure 1: Addition of 5 µl of DNA to Truenat MTB chip



The assay involves three main steps based on two main components of the Truelab® Real Time micro PCR system and three reagent packs. All reagents and consumables required for the test procedures are provided by the manufacturer, with the exception of personal protective equipment and hypochlorite-based disinfectant.

Sample preparation will take 8-10 minutes, using the liquefaction and lysis buffers (Trueprep AUTO MTB Sample Pre-treatment Pack).
Extraction and purification of DNA will take 20 – 25 minutes, using the Trueprep AUTO v2 Universal Cartridge Based Sample Prep Kit and the Trueprep AUTO v2 Universal Cartridge Based Sample Prep Device.
PCR amplification and fluorescent probe-based detection of MTB will take around 40 minutes, using Truenat chips, a Truepet® 6µlPrecision Micropipette and the Truelab micro PCR Analyzer.
At the end of the run, the "Result" screen for the Truenat MTB test indicates whether MTB has been "DETECTED" or "NOT DETECTED". For the Truenat MTB test, if MTB is detected, the estimated number of bacteria in terms of colony forming units per ml (CFU/ml) in the original sample is also reported. For the Truenat MTB Plus test, the MTB DETECTED results are described as high, medium, low or very low.
PCR amplification and fluorescent probe-based detection of rifampicin resistance will take 60 minutes, using Truenat MTB-RIFDx chips, a 6µl TruepetPrecision Micropipette and the Truelab micro PCR Analyzer.
If MTB is detected in a sample, a portion of the same DNA elute can be used to test for rifampicin resistance using a Truenat MTB-RIF Dx chip. Repeat the procedures in Step 3 above, selecting "MTB RIF" as the test type in the Truelab micro PCR Analyzer. Automated PCR amplification and fluorescent probe-based detection of rifampicin resistance takes an additional 60 minutes, resulting in a total runtime of ˜2 hours to detect MTB and rifampicin resistance.[5]

RESULTS

GENDER WISE DISTRIBUTION OF TUBERCULOSIS PATIENTS

Out of 93 male patients 51 (67%) male and among 54 female patients 25 (33%) were detected as infected with Mycobacterium tuberculosis by Truenat

Figure 2: Sex wise distribution of Tuberculosis patientsSputum microscopy smear

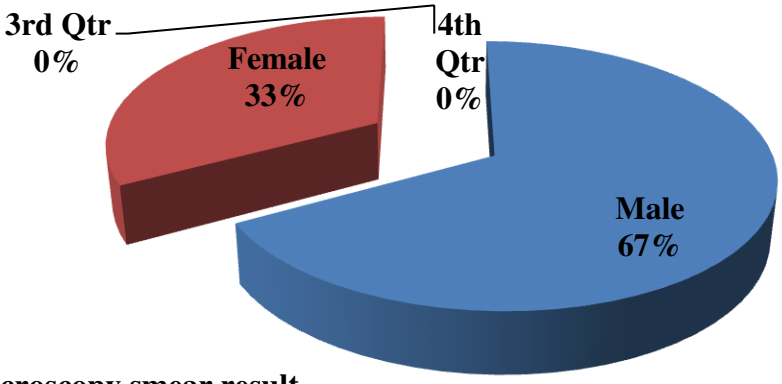


Figure 3: Sputum microscopy smear result

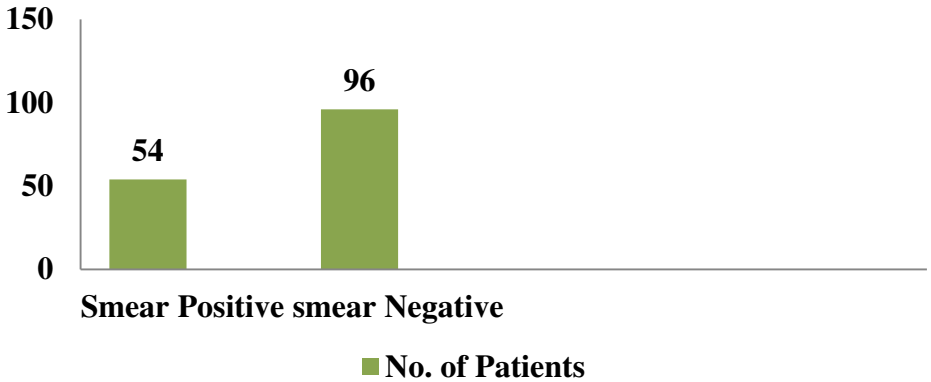


Table 1: MTB detection rate by Truenat

MTB	No. of Patients	Percentage
MTB detected	69	46%
MTB not detected	81	54%

Above are the results of samples after process for Truenat MTB detected or not

Table 2: Comparison of both the diagnostic methods

MTB	No. of Patients	Percentage
Smear positive	54	36%
Truenat positive	69	46%

Above results we get when we compare both diagnostic facilities 15 samples showing negative result for microscopy but were detected by Truenat.

Table 3: Rate of Rifampicin sensitivity by Truenat

Sensitivity	No. of Patients	Percentage
RIF sensitive	62	89.9%
RIF resistant	7	10.1%

Above are the results of rifampicin sensitivity testing done by Truenat

DISCUSSION

A major WHO priority for TB diagnostics is to implement a rapid, sputum-based molecular test to replace smear microscopy at the peripheral level (i.e., microscopy centres and attached primary healthcare facilities). The WHO's target product profile (TPP) of the "smear replacement test" includes a set of minimal and optimal requirements.[6,7] Truenat fits many minimal TPP standards, including battery-powered operation and < 2 hours to result. Truenat was evaluated earlier in India by Hinduja Hospitals, Mumbai. Sputum samples from 226 presumptive TB patients were tested by smear, culture, Truenat and an in-house PCR. A CRS including smear, culture, clinical findings and response to treatment was used to evaluate Truenat with the in-house PCR as a comparator. Truenat demonstrated a sensitivity of 91.1 per cent (CI: 86.1-94.7%) and a specificity of 100 per cent (CI: 90.0-100%) in comparison with the CRS.[8]

The results of this study show that the male (67%) to female (33%) ratio in patients of pulmonary tuberculosis is 2:1, which is in concurrence with other report by Sukhesh Rao.[9]Smear positivity in present study is about 36% (54 of 150). In a study by Deivanayagam et al, it was 15%. In a study by Kleen et al, it was found to be 29%. The increase in number of sputum samples will tend to increase the yield of smear positivity. Efficacy is dependent on skill of laboratory technician, resulting in a broad range of sensitivities and specificities reported in international studies, 25.3–81.6% and 83.4–99%.[10]

Out of 150 patients 46% were detected as MTB detected and 54% patients were MTB not detected by Truenat which is more than detected by smear examination (36%) as compared with the study by Sameera Akhtar et al. out of total 175 patients 148 patients were smear positive while 162 patients were Tuenat positive.[11]

In our study, amongst truenat positive patients, 10 % were detected as Rif resistant as shown in study by Jaykaran Charan et al, among the mixed patients (new as well as previously treated tb patients) rate of rifampicin resistance was 11.2%.[12]

CONCLUSION

The work was done to find out ideal, feasible and easy operation diagnosis methods for diagnosis of drug resistant tuberculosis. Among the methods which were used for the diagnosis of drug resistant tuberculosis, Truenat was advantageous as it could detect more cases which are missed by other conventional smear methods.The detection rate of rifampicin resistance tuberculosis among the positive cases was 10.1%. As the rate of drug resistance tuberculosis is in increasing trend among patients, it is essential to use a rapid method which can detects M.tb and rifampicin resistance simultaneously. Thus truenat is the best method in the diagnosis of pulmonary tuberculosis. Earlier detection can reduce the death rate among patients and prevent the spread of tuberculosis in the community.

CONFLICT OF INTEREST

None

Source of Support

Nil

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