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ORIGINAL ARTICLE

Diagnostic evaluation of Tru-Nat MTB/Rif test in comparison with microscopy for diagnosis of pulmonary tuberculosis at tertiary care hospital of eastern Uttar Pradesh

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ABSTRACT

Background and Objectives: This study evaluated the efficacy of the TrueLab™ Real Time mini-PCR system in providing rapid and accurate diagnostic results for tuberculosis (TB) detection in India. The goal is to improve case detection and accelerate treatment in settings with limited resources.

Materials and Methods: This prospective study was conducted by the Department of Microbiology on 120 patients, age ranging from >=15 years with at least two clinical symptoms of pulmonary TB. Molbio and Universal Cartridge Based Sample Prep were the 2 methods used for processing sputum samples. The diagnosis was based on the MTB Real Time PCR test, which has a detection limit of 100 CFU/mL. Patients under 15 years, samples lacking clinical background, saliva specimens or extra-pulmonary TB cases were excluded from the study.

Results: A total of 44.17% samples were positive for TB with maximum positivity in the age group 31-45 years. Positivity rate was found to be higher in females. In 4.17% of cases there was rifampicin resistance, which was significantly high in previously treated cases. Comparison of Truenat with Ziehl-Neelsen and fluorescent method revealed that it was more sensitive and less time consuming.

Conclusion: Truenat MTB/RIF is a sensitive detection system for TB with rapid results, which serves as an important tool in the early management of tuberculosis patients and drug-resistant-TB cases.

Keywords: Microscopy; Diagnostic accuracy; Point-of-care testing; Tuberculosis; Rapid testing; Infectious diseases

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 is 0.6 lakhs. India is the highest TB burden country. 1/4th of the global annual new cases occurs 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

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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 result. Automated methods and Molecular techniques such 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 days (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 (GeneXpert) 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 center 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 Bigtec 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 is used for LOD (Limit of Detection) determination. LOD is determined to be 100 CFU/ml in sputum sample. There is scarcity of reliable information from this Gorakhpur district.

The aim of this study was Diagnostic evaluation of Tru-Nat MTB/RIF Test in comparison with microscopy for diagnosis of pulmonary tuberculosis and the objectives include: 1) To know the prevalence of Tuberculosis in Gorakhpur district by using chip based Truelab™ Real Time micro–PCR System; 2) To understand the demographics of TB and 3) To know the prevalence of Rifampicin resistance among New and Previously treated cases.

MATERIALS AND METHODS

Study design. Hospital based prospective study

Study setting. Department of Microbiology

Study period. June 2022 to July 2022

Sample size. 120 specimens, calculated by Cochrane formula: n= Z2α/2.pq d2

Inclusion criteria. >15 years old

A minimum of two of the symptoms listed below must be present in order for pulmonary tuberculosis to be clinically suspected. The symptoms of pulmonary tuberculosis include fever, a chronic cough, weight loss, a rise in nighttime temperature, pleuritic chest pain, hemoptysis, and an abnormal chest radiograph and HIV infection in all suspected TB patients.

Exclusion criteria. Less than 15 years of age, samples obtained with no clinical background, salivary samples, all extra pulmonary cases

Sample collection method. According to RNTCP recommendations, patients were encouraged to provide an early-morning sample of profoundly expectorated sputum (12). Sputum samples were taken in sterile, leak-proof, wide-mouthed, transparent plastic containers. Ziehl-stain, Neelsen's fluorescent stain, and Truenat were applied to samples in the morning to get a high yield of positive in light of the buildup of secretions that were concentrated with bacilli.

Ziehl-Neelsen's staining. Ziehl-stain Neelsen's was used to stain the fixed smears, and an oil im-

mersion microscope was used to see them at a 100x magnification. Each slide was inspected for 5 to 10 minutes, resulting in 300 fields being looked at, and the results would be evaluated in accordance with industry standards. Samples that tested positive and negative for AFB underwent fluorescent stain and Truenat analysis (Fig. 1).

Fluorescent staining. This method involved making a smear from the material and staining it with the fluorescent dye auramine (13). When exposed to UV light, the auramine stain penetrates the bacterial cell wall and causes the cells to shine against a dark backdrop. Mycobacterium was discovered in the smear by microscopic analysis using a low power objective as bright yellow-white-like bacteria (Fig. 2).

Truenat test. Each sample underwent testing in accordance with the manufacturer's recommendations.

Sample processing procedure. According to the Molbio sample pre-treatment technique, all the samples were handled. 0.5 mL of the sediment from the centrifuged samples was added to a lysis buffer tube after the supernatant was discarded (14). The homogenised material was given a five-minute treatment with a liquefaction buffer before being placed in a Lysis buffer tube. Five minutes were spent vortexing the tube.

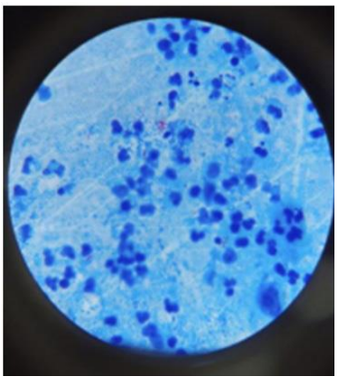

Fig. 1. Ziehl Neelsen staining

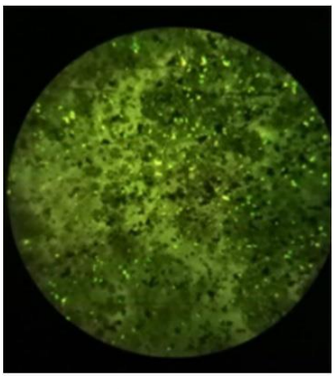

Fig. 2. Fluorescent staining

Extraction procedure. Using a kit and technology called the Universal Cartridge Based Sample Prep (Molbio diagnostics private limited, India), DNA was extracted from the samples. The pre-treated sample was then moved to the cartridge's sample chamber and inserted into the apparatus. The full elute was then aspirated out into the Elute Collecting Tube from the elute chamber (ECT).

MTB real time PCR. 6 µL of ECT purified DNA was transferred to a microtube containing freeze-dried PCR components. The lyophilized master-mix was then loaded to the MTB microchip, and a pre-programmed profile on the Truelab Analyzer was used to perform real-time PCR (15) (Fig. 3). For negative samples, the Real-time PCR results were given as "not detected" or "detected with number of Colony Forming Units per millilitre (CFU/mL)" for positive samples, and uncertain for negative samples. The MTB test's Limit of Detection (LoD) was 100 CFU/mL, and an MTB-RIF follow-up test for rifampicin resistance was scheduled to be carried out.

RESULTS

Table 1 shows age wise distribution. Out of 120 samples, 53 samples (44.17%) were positive for MTB. Highest number of positivity was seen in the age group of 31-45 years (50%), followed by 15-30 years (48.72%) 45-60 years (42.86%) and >60 years (35.48%) respectively.

Table 2 shows gender wise distribution: Highest number of samples were in males (52.5%) while in female it was seen in 47.5%. Percentage positivity was seen more in females (45.61%) as compared to males (42.86%).

Table 3 shows comparison of ZN Staining, Fluorescent Staining and Truenat MTB test. Out of 120 samples, 43 samples (35.83%) were identified as positive using either ZN staining or fluorescent staining while 67 samples (55.84%) were identified negative by both the staining methods. Overall sensitivity of ZN staining and Fluorescent staining methods were 81.3% and specificity was 100%.

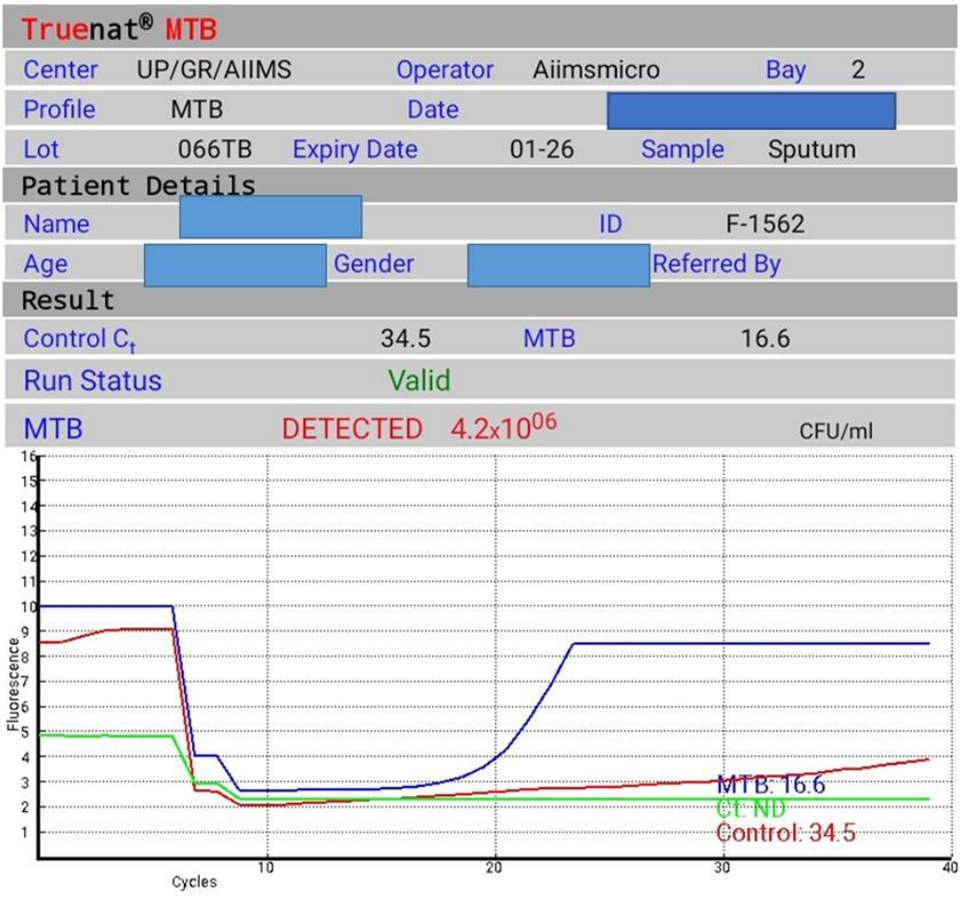

Fig. 3. RT-PCR program for MTB detection and rifampicin resistance

Table 1. Age wise distribution and percentage positivity

Age group (years)	Number of samples	No. of MTB positive samples	Percentage of MTB positive samples
15-30	39	19	48.72%
31-45	22	11	50%
45-60	28	12	42.86%
>60	31	11	35.48%
Total	120	53	44.17%

Table 2. Gender wise distribution

Gender	Number	Percentage	No of Truenat MTB samples	Percentage
Male	63	52.5%	27	42.86%
Female	57	47.5%	26	45.61%
Total	120	100%	53	44.17%

Using Tru-Nat MTB, out of 120 samples, 53 samples (44.16%) were identified as positive, which included the 8.33% which were identified negative by ZN or fluorescent staining. Overall sensitivity and specificity of Tru-Nat MTB was found to be 100%.

Table 4 shows drug resistance to rifampicin in TB diagnosed by Truenat RIF. Drug resistance to rifampicin was seen in 5 samples (4.17%), while it was intermediate in 8 samples (6.67%). No resistance was detected in 40 (33.33%) samples. Out of 5 resistance cases detected, 3 cases (60%) were previously treated cases, while 2 cases (40%) were new cases.

DISCUSSION

Due to its propensity for transmission, morbidity, and death, tuberculosis is a highly contagious illness that poses a serious danger to India's public health (15). Tuberculosis is a serious public health concern in India despite the availability of cutting-edge diagnostic techniques and a range of treatment options,

Table 3. Comparison of ZN staining, fluorescent staining, and Tru-NAT MTB test results

Test method	True positive	True negative	False positive	False negative	Total samples	Sensitivity (%)	Specificity (%)
ZN staining	43	67	0	10	120	81.13	100
Fluorescent staining	43	67	0	10	120	81.13	100
Tru-Nat MTB	53	67	0	0	120	100	100

Table 4. Drug resistance to rifampicin in Tb diagnosed by Tru-Nat Rif

	Tru-NAT RIF Test			
	Detected (%)	Not Detected (%)	Indeterminate (%)	Total (%)
Drug resistance	5 (4.17%)	40 (33.33%)	8 (6.67%)	53 (44.17%)
No resistance	0	67 (55.83%)	0	67 (55.83%)

with negative social and economic repercussions. Although culture is preferred as the standard method for diagnosis, this study compared the Truenat MTB/RIF Test's diagnostic performance to that of microscopy in a resource-constrained environment, such as our institute, where culture facilities are not accessible.

120 people with records made comprised the research group. Out of 120 people, the age range with the highest percentage of positive was 31-45 years, with 50%; 15-30 years, with 48.72%; 45-60 years, with 42.86%; and above 60 years, with 35.48% (Table 1). It is obvious that a sizable portion of the positive population was in the young and fertile age bracket (16). Lastly, 35.48% of those with more than 60 years of age showed positive. This group typically has co-occurring systemic illnesses, which puts patients at risk for disease complications, disease dissemination, and treatment challenges due to associated side effects. Distribution by gender is seen in Table 2. Males (52.5%) had the highest percentage of samples, while females (47.5%) had the lowest percentage. Nonetheless, ladies (45.61%) showed somewhat greater percentages of optimism than males (42.86%). This has significant consequences for the methods used to combat TB (Tuberculosis Research Centre, 2003). Females in the reproductive age group are more likely to: (a) transmit infections to their offspring, which is sometimes attributed to nursing; (b) experience complications during pregnancy and after delivery due to antenatal and postpartum morbidity; and (c) default on payments because of various factors, including working from home, caring for children, inconvenience from visiting a DOTS centre (17).

The results were the same for men as well. Due to many socioeconomic factors, such as men being the only breadwinners, a higher likelihood of employment in unorganized industries, and a lower likelihood of disease knowledge, default is more likely in developing and high incidence nations. Anecdotal information also shows that due to their job schedules, guys in the reproductive age group have found it difficult to visit DOTS clinics. It is important to consider each of these factors and create the best possible tactics. Table 3 compares Truenat, fluorescence staining, and ZN staining. Out of 120 samples, 43 samples (or 35.83%) were positive by Truenat and Ziehl Neelsen staining, whereas 67 samples (or 55.84%) were negative. However using Tru-Nat MTB test, out of the tested 120 samples, 53 samples (44.16%) were found to be positive. Notably, 8.33% of samples which were found negative through staining methods were found to be positive through Truenat MTB test. The usefulness of this novel method in India was demonstrated by the present study's strong concordance for versus microscopy. According to previous studies, Truenat MTB/RIF has greater sensitivity and specificity than microscopy (18). While conducting research it was found that smear microscopy has a sensitivity of 63% and a specificity of 100%, but Truenat has a sensitivity of 91% and a specificity of 100%. In different research, when compared to culture, the Truenat had a sensitivity of 92.9% for samples with positive smear results and a sensitivity of 75% for samples with negative smear results. After conducting research, it was also found that Truenat MTB has sensitivity of 73% and specificity of 98% when compared with culture.

Moreover, Truenat had a sensitivity of 100% and a

specificity of 95.1% when compared to microscopy, which had a sensitivity of 100% and a specificity of 96.6% when culture was used as the gold standard test (19). Moreover, research revealed that the sensitivity and specificity of Truenat MTB are 88% and 97.2%, respectively.

In Table 4 Drug resistance to rifampicin in TB diagnosed by Truenat RIF is shown. Rifampicin drug resistance was present in 4.17% of patients, whereas it was intermediate in 6.67% of cases. In 33.33% of instances, no resistance was found. In research 113, 19% of the samples had rifampicin resistance (20). According to the study, of the 53 positive cases, 47 (88.67%) had only recently been identified, while only six (11.32%) had already been treated.

Rifampicin resistance was found in 5 instances out of 53 positive cases, 3 of which (60%) had previous treatment and 2 of which (40%) were brand-new cases. It was found that 13.59% resistance in fresh patients and 25.92% resistance in those that had already been treated. To eradicate tuberculosis by 2025, a key obstacle is drug-resistant tuberculosis (DRTB). The prevalence of MDR-TB was 2.84 percent among newly diagnosed cases and 11.6 percent among those who had already received treatment, according to the Drug Resistance Study conducted in India in 2014-16 (21).

CONCLUSION

Tuberculosis (TB) continues to be a major global public health problem, especially in resource-constrained countries such as India. This study was conducted to assess the diagnostic utility of Tru-NAT MTB/RIF test for detection of pulmonary TB in comparison with conventional microscopy. The 31-45 age group had the greatest frequency of TB-positive cases. A higher proportion of women than men tested positive, highlighting a need for targeted interventions in specific age and focused demographics. Relatively, Tru-NAT MTB/RIF had sensitivity significantly better than microscopy, indicating its superior ability to detect TB accurately, including in cases with low bacterial loads. The fast turnaround-time and high sensitivity of Tru-NAT MTB/RIF exhibits potential application for early detection and management of TB. Tru-NAT MTB/RIF identified rifampicin resistance in 4.17% of cases, which is crucial for guiding appropriate treatment regimens, particularly in previously treated patients. As a simple, reliable and rapid assay for TB detection, the Tru-NAT MTB/RIF kit is highly adaptable to decentralized testing in resource-poor settings without advanced laboratory facilities. Further studies should be conducted for assessing the contributory role of Tru-NAT MTB/RIF in TB control programs especially in terms of its cost- effectiveness, scalability and incorporation into existing healthcare systems on a larger scale.

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