British Journal of Medicine & Medical Research
8(9): 772-780, 2015, Article no.BJMMR.2015.505
ISSN: 2231-0614

SCIENCEDOMAIN *international*
www.sciencedomain.org

Utility of GenoType MTBDR*Plus* Assay for Direct Detection and Drug Susceptibility Testing of *Mycobacterium tuberculosis* from Probable Tuberculous Meningitis Patients

Gupta Renu[1*], Thakur Rajeev[1], Jalan Nupur[1], Rawat Pumanshi[1], Kushwaha Suman[2] and Paul Mousumi[1]

[1]Department of Microbiology, Institute of Human Behavior and Allied sciences, Dilshad Garden, Delhi 95, India.
[2]Department of Neurology, Institute of Human Behavior and Allied sciences, Dilshad Garden, Delhi 95, India.

Authors' contributions

This work was a collaborative effort of all the authors. Author GR provided conceptual and technical guidance in designing and executing the study. She wrote the manuscript. Author TR critically reviewed the manuscript. Author JN managed the literature search, statistical analysis and processed all CSF samples by GenoType MTBDR assay. Author RP carried out the DNA extraction and PCR of all the clinical samples. Author KS helped in enrolling patients after thorough clinical work up. Author PM did smear Microscopy, culture and drug susceptibility testing of M. tuberculosis isolates. All authors have read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2015/18176
Editor(s):
(1) Xin-an Liu, Neuroscience Department, The Scripps Research Institute, Scripps, Florida, USA.
Reviewers:
(1) Guadalupe García-Elorriaga, Hospital de Infectología, CMNR, Mexico.
(2) Ketan Vagholkar, General Surgery, D. Y. Patil University, India.
(3) Mathew Folaranmi Olaniyan, Department of Medical Laboratory Science, Achievers University, Owo, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1121&id=12&aid=9440

Original Research Article

Received 9th April 2015
Accepted 2nd May 2015
Published 27th May 2015

ABSTRACT

Aims: To evaluate GenoType® MTBDR*plus* line probe assay as a diagnostic tool for detection of *Mycobacterium tuberculosis* and drug susceptibility testing from cerebrospinal fluid of probable tuberculous meningitis patients.
Study Design: A prospective, double blind study.
Place and Duration of study: Dept. of Microbiology and Neurology, Institute of Human Behavior

**Corresponding author: Email: renugoyal_123@yahoo.co.in;*

and Allied sciences, Delhi, India between February 2014 to October 2014.
Methodology: Cerebrospinal fluid collected from 107 probable meningitis patients with diagnostic score >10 were subjected to smear microscopy, automated liquid culture (BACTEC MGIT 960) and Polymerase chain reaction (IS6110). All the samples were also subjected to GenoType® MTBDR*plus* line probe assay for detecting M. tuberculosis and drug susceptibility. Drug susceptibility testing of all the *M. tuberculosis* isolates was done by BACTEC MGIT 960 and GenoType® MTBDR*plus* line probe assay.
Results: The sensitivity, specificity of the assay for *M. tuberculosis* detection was 49.5%, 100% against clinical diagnosis as reference standard and 68.9%, 100% against definitive diagnosis as reference standard. A diagnostic accuracy of 56.8% (kappa 0.22), 75% (kappa 0.46), were seen in patients with probable and confirmed diagnosis respectively. The drug susceptibility results for Isoniazid and Rifampicin could be delineated in only 39.2% of patients.
Conclusion: This assay proved to have better sensitivity, diagnostic accuracy than smear microscopy and automated liquid culture for early detection of *M. tuberculosis* from probable tuberculous meningitis patients and has comparable sensitivity to culture (39.2%) for detection of drug susceptibility (though on different isolates). Rapid turnaround time and user friendliness makes it an acceptable assay for simultaneous early detection of *M. tuberculosis* and its drug susceptibility for better patient management.

Keywords: GenoType MTBDRplus assay; drug susceptibility testing; nucleic acid amplification; cerebrospinal fluid.

ABBREVIATIONS

NAAT: Nucleic acid amplification test

DEFINITION:

Definite Tuberculous Meningitis

*Clinical entry criteria: Symptoms and signs of meningitis including one or more of the following: headache, irritability, vomiting, fever, neck stiffness, convulsions, focal neurological deficits, altered consciousness, or lethargy **plus**; One or more of the following; Acid-fast bacilli seen in the CSF; Mycobacterium tuberculosis cultured from the CSF; CSF positive by nucleic acid amplification test.*

Probable Tuberculous Meningitis

*Clinical entry criteria as above **Plus**; A total diagnostic score of 10 or more points (when cerebral imaging is not available) or 12 or more points (when cerebral imaging is available) plus exclusion of alternative diagnosis. At least 2 points should either come from CSF for cerebral imaging criteria.*

1. INTRODUCTION

Tuberculous meningitis (TBM) is the most distressing complication of tuberculosis and accounts for approximately 7-12% of tuberculosis cases in developing world [1]. This disease is associated with a very high mortality (30%) in cases of infection with fully sensitive organism and much higher mortality (60%) and morbidity in infection with drug resistant organisms and Human Immunodeficiency Virus co infected individuals [2,3]. Delay in diagnosis and initiation of treatment further worsens the clinical outcome [3,4]. Precise and rapid diagnosis of TBM and drug resistance in useful clinical time frame can not only improve the survival in these patients but also prevent devastating neurological squeal.

The confirmed microbiological diagnosis in useful clinical time frame is still not possible due to paucibacillary nature of the disease [3-5]. The conventional diagnostic tests besides suffering from low sensitivity takes minimum of 2-3 weeks for detection of *M. tuberculosis* followed by another 2-3 weeks for drug resistance testing resulting in loss of the most valuable time for evidence directed therapy [3-5] Nucleic acid amplification tests (NAAT) as diagnostic tests for TBM has given a highly variable sensitivity on commercial assays and are notoriously difficult to

standardize and implement in-house with adequate quality controls in resource limited high burden microbiology laboratories in developing world [5,6]. There is an immense need of a sensitive, affordable, user friendly assay capable of giving rapid reliable results.

GenoType® MTBDR*plus* line probe assay (HAIN Life Sciences, Germany) based on DNA strip technology is approved by World Health Organization for direct detection of multi drug resistant (MDR) *M. tuberculosis* from smear positive/negative pulmonary specimens and *M. tuberculosis* cultures after evaluation by the Foundation for Innovative new diagnostics (FIND) and has become available in many laboratories across the developing countries [7]. This assay permits the molecular identification of the *M. tuberculosis* complex and resistance to Rifampicin (RIF) and Isoniazid (INH) and is based on multiplex PCR followed by reverse hybridization for detection of deletion in wild type gene loci and mutation in rpoB (RNA polymerase B subunit), katG (catalase peroxidase) and inhA (inoyl coenzyme A reductase) loci. This test is affordable and has become available in many high burden diagnostic laboratories for pulmonary tuberculosis and is user friendly with minimum staff training [8,9]. However this test has not been sufficiently evaluated for direct detection of *M. tuberculosis* and drug resistance from extra pulmonary patients [10].

In a preliminary study, we evaluated this test for detection of *M. tuberculosis* and drug susceptibility in few confirmed TBM patients [11]. The present study is an attempt to assess sensitivity, specificity of this assay for detection of *M. tuberculosis* and drug susceptibility in probable TBM patients presenting to a tertiary care neuropsychiatry hospital.

2. MATERIALS AND METHODS

Ethical approval for the study was obtained from the Institutional Ethical Review Committee for Biomedical Research. Informed written consent for lumbar puncture was obtained from all patients or their relatives before inclusion in the study.

2.1 Patient Selection

This prospective study was conducted on probable TBM patients presenting to a tertiary care neuropsychiatry hospital of Delhi from Feb 2014- Oct 2014. All the consecutive patients presenting with clinical suspicion of chronic meningitis with symptoms of headache, irritability, vomiting, fever, neck stiffness, convulsions, focal neurological deficits, altered consciousness, or lethargy for more than 5 days were screened for CSF cytology, biochemistry and/or cerebral imaging and for evidence of tuberculosis elsewhere as per criteria laid by Marias et al. [12]. The patients were recruited for this study only if they had diagnostic score ≥10 (when cerebral imaging was not done) or ≥12 (when cerebral imaging was done) using validated scoring system [12]. In addition 18 non TBM patients with non infectious neurological disorder were included as negative controls. Patients with significant co-existing disease like pre-existing neurological deficit, seizure disorder, mental retardation, cerebral palsy or already on antiuberculous drug treatment were not included in the study. Any patient diagnosed to have any other cause of meningitis was excluded later from the study.

Approximately 3-5 ml of CSF sample was received in Microbiology department for TBM diagnosis. The sample was processed as per standard microbiological techniques for microscopy and culture in BACTEC MGIT 960 (MGIT 960; Becton Dickinson Systems, sparks, MD) by the staff well trained in doing these procedures [13,14]. PCR and GenoType MTBDR*plus* assay were performed by two different personnel who were blinded to microscopy, culture and results of each other.

2.2 DNA Extraction and PCR for *M. tuberculosis*

1 ml of CSF sample was incubated overnight at 65°C with 20 ul of Proteinase K (10 mg/ml) followed by boiling for 10 min [1]. DNA was stored at -20°C till further testing. Conventional PCR for amplification of IS6110 gene was done using forward primer 5'-CCTGCGAGCGTAGGCGTCGG-3' and reverse primer 5'- CTCGTCCAGCGCCGCTTCGG-3' as: Initial denaturation at 95°C for 4 min followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 30 sec. A final extension was done at 72°C for 7 min. Amplified gene product were analyzed using agarose gel (1.5%) electrophoresis.

2.3 GenoType MTBDR*plus* Assay

DNA was extracted using Genolyse kit (HAIN Life Sciences, Germany). Multiplex PCR for the

detection of drug resistance genes (rpoB, katG, inhA) was performed using 35 µl of primer nucleotide mix, 10 µl of Taq DNA polymerase-PCR buffer mix and 5 µl of supernatant in a final volume of 50 µl. Amplification was done in a thermal cycler (PalmCycler, Genetix Biotech Asia Pvt. Ltd) using cycling parameters as: Initial denaturation of 15 min at 95°C, followed by 20 cycles of 30 sec at 95°C and 2 min at 65°C, and 30 cycles of 25 sec at 95°C, 40 sec at 50°C and 40 sec at 70°C and the extension step of 8 min at 70ºC. Reverse hybridization was performed using Twincubator (HAIN Life Sciences, Germany) by GenoType MTBDR*plus* kit as per manufacturer's instruction [15]. The test was interpreted as positive for *M. tuberculosis* complex if a clear *M. tuberculosis* complex control (TUB) was seen along with conjugate control and amplification control bands and negative if MTB control band was faint or absent. The absence of any of the wild-type bands or presence of any mutation band implied resistance in presence of clear control bands. A positive (H37Rv) and negative (water) control was run in every assay.

2.4 Drug susceptibility testing (DST)

For all the culture isolates was done by BACTEC MGIT 960 using standard critical concentration for INH (0.1 ug/ml) and RIF (1 ug/ml) as well as by GenoType MTBDR*plus* assay [13].

2.5 Statistical Methods

The sensitivity, specificity, positive predictive value, negative predictive value was calculated using defined formula against two reference standards 1) Definitive diagnosis of TBM (microscopy and/or culture and/or PCR) and 2) probable diagnosis [16]. The sensitivity, specificity of microscopy, culture and PCR was calculated against probable diagnosis as reference standard. The exact 95% confidence interval was calculated using Graph pad calculator [17].

3. RESULTS

A total of 113 patients fulfilled the enrollment criteria for the study. 6 patients were excluded later as two samples were positive for Cryptococcus, 1 grew Non Tuberculous Mycobacteria, 3 samples were contaminated. Thus 107 patients were included in final analysis (Fig. 1).

3.1 Microbiological Diagnosis

The sensitivities of smear, culture and PCR for *M. tuberculosis* detection were 6.5% (7/107; 95% CI, 2.6% to 13%) 39.3 %(42/107; 95% CI, 29.9% to 49.1%) and 65.4% (70/107; 95% CI, 55.6% to 74.3%) respectively against Probable TBM as reference standard. The definitive diagnosis's of TBM was made in 69.1% patients (74/107; 95% CI, 59.5 to 77.7) by microscopy and/or culture and/or PCR. All the control patients were negative by microscopy, culture and PCR. Fig. 1 depicts the results of CSF processing.

3.2 GenoType MTBDR*plus* Assay

The sensitivity/specificity of GenoType MTBDRplus assay for *M. tuberculosis* detection was 49.5% (53/107; 95% CI, 39.7 to 59.3)/100% against probable diagnosis as reference standard and 68.9% (51/74; 95% CI, 57.1 to 79.1)/100% against definitive TBM as reference standard. In addition, this assay could detect *M. tuberculosis* in 2 probable TBM patients who were microscopy, culture and PCR negative. The diagnostic efficacy of this assay is shown in Table 1.

3.3 Drug Susceptibility Testing

DST of *M. tuberculosis* culture isolates was done by BACTEC MGIT 960 and GenoType MTBDR*plus* assay in only 42 isolates as 32 patients were PCR positive but culture negative. For CSF samples directly tested by GenoType MTBDR*plus* assay, all the six locus control bands were present in only 42 of the 53 TUB band positive samples thus enabling detection of INH and RIF susceptibility in 42 clinically diagnosed patients (24 culture positive, 18 culture negative). However, RIF susceptibility was interpretable in all the 53 TUB band positive samples whereas in 11 TUB band positive samples inhA control band was missing. By GenoType MTBDR*plus* assay only one sample was detected as MDR and 3 samples were mono resistant to INH (out of 42).

The result of all the three assay i.e MGIT 960 DST, GenoType MTBDR*plus* assay from culture and direct CSF sample were available for only 31 samples as 22 clinical samples tested positive by GenoType MTBDR*plus* assay were culture negative (Table 2). Out of 31 samples, 7 samples had missing inhA control band and there was complete agreement by all the three methods for 23 strains (74.1%): 20 strains were sensitive, one MDR and two mono resistant to INH. One *M.*

tuberculosis isolate was tested resistant to INH by MGIT 960 but was sensitive by GenoType MTBDR*plus* assay both on direct CSF sample and culture isolate (Table 2).

Table 1. Diagnostic utility of GenoType MTBDR*plus* assay

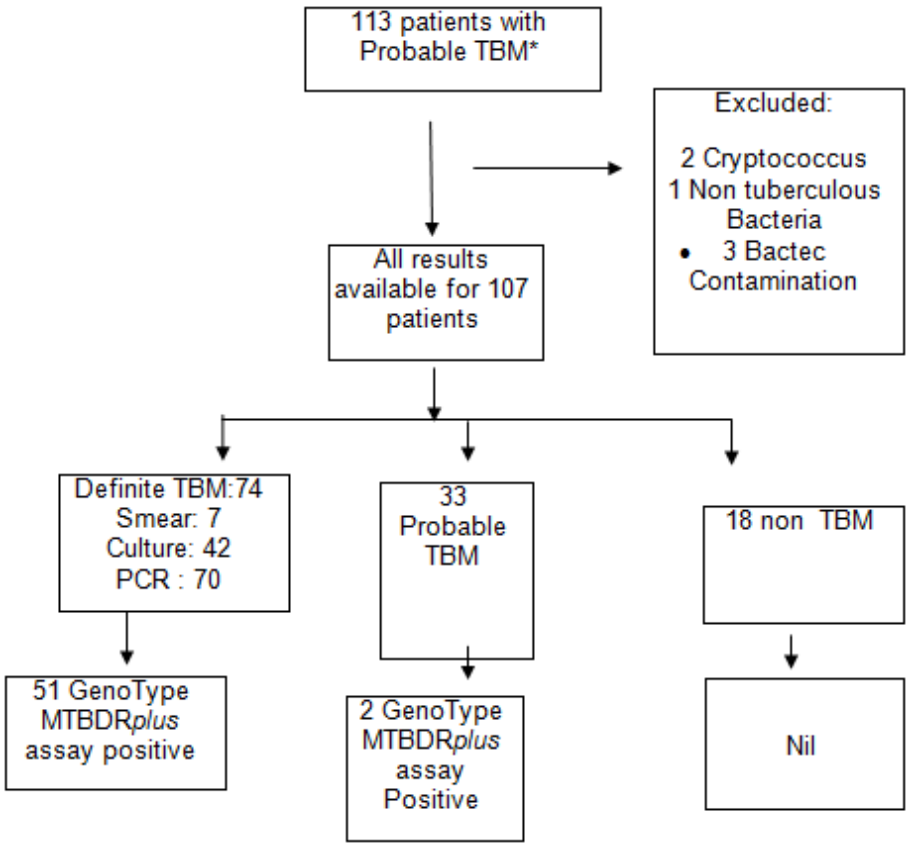
Test	Category	Sensitivity %	Specificity %	PPV%	NPV%	Accuracy%	Kappa
Gentoype MTBDR plus assay	Confirmed TBM	68.9	100	100	44	75	0.46 (moderate)
	Probable TBM	49.5	100	100	25	56.8	0.22 (fair)

PPV: Positive predictive value; NPV: Negative predictive value

Table 2. Comparative analysis of drug susceptibility test results by phenotypic BACTEC MGIT 960 and GenoType MTBDR assay in CSF samples and Cultures

	Phenotypic Pattern				Molecular pattern	
Mag No	BACTEC MGIT 960		HAIN Culture		CSF SAMPLE	
	RIF	INH	RIF	INH	RIF	INH
3402	S	R	S	R ΔkatG WT S315T1	S	ΔinhA CB
3417	S	S	S	R ΔinhA WT1 C15T	S	ΔinhA CB
3844	R	R	R ΔWT8,S531L	R ΔkatG WT S315T1	R ΔWT8 S531L	R Δ katGWT S315T1
2527	S	S	S	S	S	S
3941	S	R	S	R ΔkatGWT S315T1	S	R ΔkatGWT S315T1
2535	S	S	S	S	S	S
2803	S	S	S	S	S	S
2807	S	S	S	S	S	S
3327	S	S	S	S	S	S
3416	S	S	S	S	S	S
3328	S	S	S	S	S	S
3344	S	S	S	S	S	S
3345	S	S	S	S	S	S
3558	S	S	S	S	S	ΔinhA CB
3471	S	S	S	S	S	S
3608	S	S	S	S	S	S
3332	S	S	S	S	S	S
3261	S	S	S	S	S	S
2861	S	S	S	S	S	S
3385	S	S	S	S	S	S
3346	S	S	S	S	S	S
3301	S	R	S	R ΔkatGWT S315T1	S	R ΔkatG WT S315T1
3372	S	S	S	S	S	S
2967	S	S	S	S	S	S
3028	S	S	S	S	S	S
2824	S	S	S	S	S	S
3284	S	S	S	S	S	ΔinhA CB
3603	S	R	S	S	S	S
3717	S	S	S	S	S	Δ inhA CB
3757	S	S	S	S	S	ΔinhA CB
3748	S	S	S	S	S	ΔinhA CB

ΔkatG WT S315T1: deletion of katG wild type, mutation at codon 315; ΔinhA CB: deletion of inhA control band (uninterpretable)



*Marias et al: 113 patients clinically diagnosed Probable TBM

Fig. 1. Flow chart of CSF processing results of patients recruited in the study

4. DISCUSSION

TBM is a medical emergency and a large number of patients are started on presumptive treatment as microbiological confirmation by microscopy, culture is insensitive, time consuming and nucleic acid amplification tests are very difficult to standardize and maintain in resource limited settings [4-6]. Even if diagnosis of TBM is established, DST results are available only from 30-60% of patients who grow *M. tuberculosis* on culture and that too after a delay of 7-10 days after culture confirmation [4]. This leads to over utilization of already scarce resources as well as increased risk for emergence of drug resistance and poor compliance in patients. Though recently GenoType MTBDR*plus* assay has become available in large number of resource limited high burden settings in developing world for pulmonary specimens yet its utility for direct detection of *M. tuberculosis* and drug resistance for extra pulmonary clinical specimens is questionable. Only very few studies have assessed this test for detection of *M. tuberculosis* drug resistance in direct CSF samples from confirmed or probable TBM patients [10,11].

In a preliminary study, we had evaluated GenoType MTBDR*plus* assay for detection of *M. tuberculosis* and drug resistance in few confirmed TBM patients and found a sensitivity of 55% [11]. Encouraged by these findings, we went ahead to evaluate this assay on probable TBM patients. In the present study the sensitivity of GenoType MTBDR*plus* assay for *M. tuberculosis* detection was 49.5% with diagnostic accuracy of 56.8% with fair kappa of 0.22 against probable diagnosis as reference standard which was much better than sensitivity, diagnostic accuracy for detection of *M. tuberculosis* by microscopy (6.5%, 20%, kappa 0.02), culture (39.3%, 48%, kappa 0.15) but was less than PCR (69.1%, 73.6%, kappa 0.39) (Table 2). The GenoType MTBDR*plus* assay could detect *M.*

tuberculosis in 68.9% of all confirmed TBM cases (diagnostic accuracy 75%, kappa 0.46) and could also detect *M. tuberculosis* in additional 2 probable TBM patients who were microbiologically negative for *M. tuberculosis*. These two probable TBM patients were not false positive as they were radiologically proven TBM and showed a good response to anti tubercular treatment at 6 months follow up. Interestingly, GenoType MTBDR*plus* assay could not detect *M. tuberculosis* in all the smear positive, culture positive and PCR positive samples which is against the conventional wisdom as generally, the comparative higher bacterial load in smear and culture positive samples should have been easily detected by this molecular assay. On the contrary, the assay could detect *M. tuberculosis* in 2 additional samples which were smear negative, culture negative and PCR negative probable TBM patients. This suggests that for TBM diagnosis there is no single rule out test and all the tests are contingent upon their ability to pick the target in tested volume of CSF. Additionally, the paucibacillary nature of the disease and uneven distribution of bacilli due to clump formation in clinical samples can provide some more explanation for this [18]. Though GenoType MTBDR*plus* assay is not approved for detection of *M. tuberculosis* directly from clinical sample but it can be of immense benefit in establishing TBM diagnosis with certainty due to its rapid turnaround time, user friendliness and practically minimal chance of contamination [18]. Clear interpretable results for drug resistance testing (all 6 control bands) were seen in only 42 (39.2%) samples and drug susceptibility by MGIT DST could also be done in only 42 culture isolates but from different patients Fig. 2. However, additional 11 CSF samples had interpretable bands for detection of *M. tuberculosis* and RIF resistance but had missing inhA locus control band. This could be due to competitive inhibition of amplification of inhA gene in paucibacillary specimens as none of the culture isolates from these samples had missing inhA control band.

Of the 42 CSF samples interpretable by GenoType MTBDR*plus* assay, 38 samples were sensitive to both INH and RIF, one sample was MDR with mutation at codon 531 for RIF and at codon 315 INH resistance. All 3 INH mono resistant samples had mutation at codon 315. Of these 42 samples, 24 samples were culture positive and were also tested for drug sensitivity by MGIT DST and GenoType MTBDR*plus* assay. Except for one isolate, there was complete agreement in results of GenoType MTBDR*plus* assay from direct sample and culture isolate.

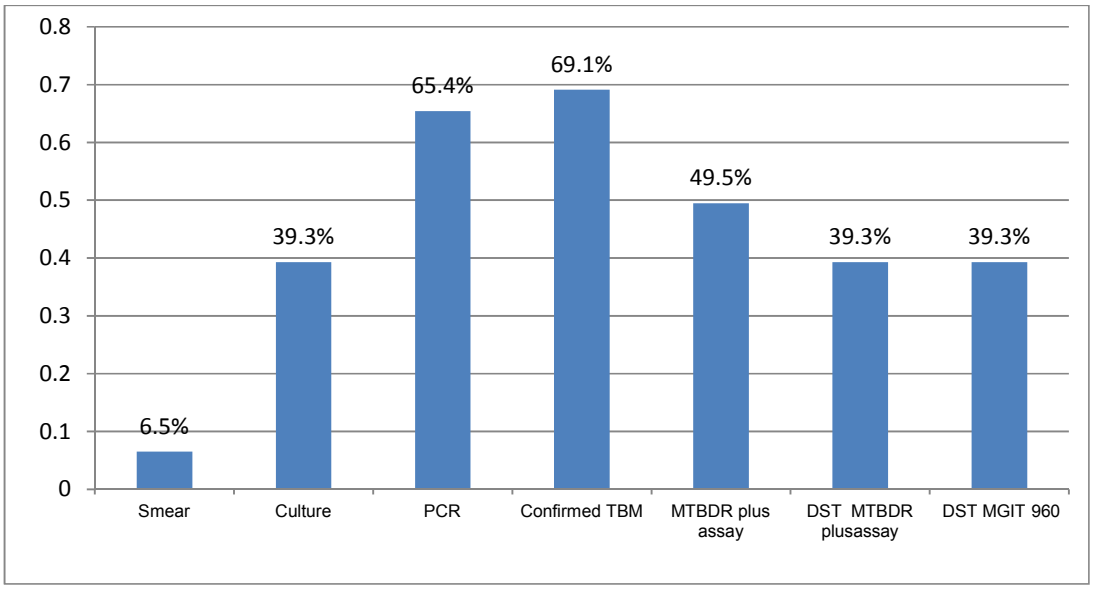


Fig. 2. Sensitivities of microbiological diagnosis and GenoType MTBDR*plus* assay for *M. tuberculosis* detection and drug susceptibility testing against probable diagnosis as reference standard. DST: Drug susceptibility testing

This isolate was resistant by MGIT DST but sensitive by Genotype MTBDR*plus* assay which could be due to some unidentified mutation in some other genomic region which is not targeted by this assay [19,20]. All the 11 TUB band positive samples were sensitive to RIF and results were similar to MGIT DST and GenoType MTBDR*plus* assay from culture. The results from our study are in contrast to previously published study from china which has shown excellent sensitivity (93.3%) for detection of drug resistance in CSF samples from confirmed TBM patients and much higher rates of drug resistance in TBM patients [10].

5. CONCLUSION

The GenoType MTBDR*plus* assay thus proved to be a rapid, easy to perform test for diagnosing TBM in probable TBM patients. The assay had much better sensitivity and diagnostic accuracy than microscopy and culture and could also detect drug susceptibility in some of the culture negative patients. The test can be used for simultaneous detection of *M. tuberculosis* and drug susceptibility directly in CSF from patients adequately screened with validated scoring criteria in high burden resource limited settings where other better platforms for early diagnosis and susceptibility testing are not available. The limiting sensitivity of this test is offset by its immense benefit of early and precise simultaneous detection of *M. tuberculosis* and drug resistance for better patient management in probable TBM patients.

We are thankful to Indian Council of Medical Research for providing financial support for conducting this study. We are thankful to Mr. Maneesh Rawat for his untiring support in collecting specimens and patient clinical details.

CONSENT

Consent for inclusion in the study was taken both in English and local language.

ETHICAL APPROVAL

The study was approved by ethical committee of IHBAS (F.1(15/Dir/IHBAS/Ethics/2011/010).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kulkarni SP, Jaleel MA, Kadival GV. Evaluation of an in-house-developed PCR for the diagnosis of tuberculosis meningitis in Indian children. J Med Microbiol. 2005;54:369-73. [PMID:15770022]
2. Caws M, Thwaites G, Stepniewska K, Lan NT, Duyen NT, Phuong NT, et al. Beijing GenoType of *Mycobacterium tuberculosis* is significantly associated with human immunodeficiency virus infection and multidrug resistance in cases of tuberculous meningitis. Clin Microbiol. 2006;44:3934-3939. [PMID: 16971650]
3. Hosoglu S, Geyik M, Balik I, Aygen B, Erol S, Aygencel TG, et al. Predictors of outcome in patients with tuberculous meningitis. Int. Tuberc. Lung Dis. 2002;6:64-70. [PMID: 11931403]
4. Ho J, Marais B, Gilbert G, Ralph A. Diagnosing tuberculosis meningitis- have we made any progress. Trop Med Int Health. 2013;18:783-793. [PMID: 23521060]
5. Murthy JM. Tuberculosis meningitis: The challenges. Neurol India. 2010;58(5):716-22. [PMID: 21045494]
6. Pai M, Flores LL, Pai N, Hubbard A, Riley LW, Colford JM, Jr. Diagnostic accuracy of nucleic acid amplification tests for tuberculous meningitis: a systematic review and meta-analysis. Lancet Infect. Dis. 2003;3:633–643. [PMID: 14522262]
7. World Health Organization. Molecular line probe assays for rapid screening of patients at risk of Multidrug Resistant Tuberculosis (MDR-TB). Policy Statement. Geneva; 2008. Available:http://www.who.int/tb/features_archives/policy_statement.pdf
8. Hillemann D, Rüsch-Gerdes S, Richter E. Evaluation of the GenoType MTBDRplus assay for rifampin and isoniazid susceptibility testing of *Mycobacterium tuberculosis* strains and clinical specimens. Clin Microbiol. 2007;45:2635-40. [PMID: .17537937]
9. Ling DI, Zwerling AA, Pai M. GenoType MTBDR assays for the diagnosis of multi-drug resistant tuberculosis. Eur Respir J. 2008;32:1165-74. [PMID: 18614561]

10. Duo L, Ying B, Song X, Lu X, Ye Y, Fan H, et al. Molecular profile of drug resistance in tuberculous meningitis from southwest China. Clin Infect Dis. 2011;53:1067-73. [PMID: 22021920]
11. Gupta R, Thakur R, Gupta P, et al. Evaluation of Geno Type MTBDR*plus* line probe assay for early detection of drug resistance in tuberculous meningitis patients in India. Global Infectious Diseases. 2015;7:5-10. [PMID: 25722613]
12. Marais S, Thwaites G, Schoeman JF, Török ME, Misra UK, Prasad K, et al. Tuberculous meningitis: A uniform case definition for use in clinical research. Lancet Infect Dis. 2010;10:803-12. [PMID: 20822958]
13. Sparks BD. Bactec MGIT SIRE Drug Kit Package Insert; 2010. Available:http://www.bd.com/ds/technicalcenter/inserts/PP118JAA(201006)/pdf
14. Mycobacteria. In: Winn W Jr, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, *et al*., editors. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Philadelphia: Lippincott, Williams & Willkins. 2006;1064-124.
15. GenoType MTBDRplus Packet Insert; 2012. Nehren, Germany: HAIN Lifesciences.
16. Beaglehole R, Bonita R, Kjellstrom T. Basic epidemiology. 2nd ed. World Health Organisation, Geneva; 2006.
17. Graph pad software. Available:http://www.graphpad.com/quickcalcs
18. Vadwai V, Boehme C, Nabeta P, Shetty A, Alland D, Rodrigues C. Xpert MTB/RIF: A new pillar in diagnosis of extrapulmonary tuberculosis? Clin Microbiol. 2011; 49:2540-2545. [PMID: 21593262].
19. Brossier F, Veziris N, Jarlier V, Sougakoff W. Performance of MTBDR plus for detecting high/low levels of *Mycobacterium tuberculosis* resistance to isoniazid. Int .Tuberc Lung Dis. 2009;13:260-5. [PMID: 19146757].
20. Yadav RN, Singh BK, Sharma SK, Sharma R, Soneja M, Sreenivas V, et al. Comparative evaluation of GenoType MTBDRplus line probe assay with solid culture method in early diagnosis of multidrug resistant tuberculosis (MDR-TB) at a tertiary care centre in India. PLoS One. 2013;8:e72036. [PMID: 24039735].

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=1121&id=12&aid=9440