Evaluation of Novel Chip Based Test - Truenat with Traditional Genexpert for Detection of Tubercular Meningitis

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AA and SS conceptualized the study. Author SS did data curation. Authors AA, SS and AK did formal analysis. Authors SS, AK and RA investigated the study. Authors AA, SS and AK performed methodology. Authors AA and AK helped as Project administration, searched for resources and supervised the study. Author SS wrote original draft of the manuscript. Authors AA, Ak and RS reviewed and edited the manuscript. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: TRUENAT is a novel chip based test developed in India. Its use for pulmonary samples has been approved by World Health Organisation. Government of India has recently approved it for Extrapulmonary cases.

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Aim: To study the role of TRUENAT in diagnosis of Tubercular Meningitis(TBM) and the co-relation between TRUENAT and GeneXpert.

Study Design: Prospective study.

Subjects and Methods: 75 Children and adolescents with strongly suggestive TBM as per NTEP guidelines [1] were enrolled. Clinical, Radiological and Cerebrospinal fluid(CSF) analysis were carried out. CSF was tested by TRUENAT and GeneXpert.

Results: TRUENAT detected 13/75 cases in comparison to GeneXpert which detected 16/75 cases. Out of these, in only 4 samples MTB was detected by both these tests. Concordance between the two tests was 72%. Cohen kappa (k-0.105) analysis showed slight agreement between the two tests. Higher CSF count(>10cell) was associated with higher chances of positivity in GeneXpert as well as TRUENAT.

Conclusion: TBM diagnosis requires a combination of clinical, radiological and biochemical parameters. Molecular tests alone cannot detect bacilli in many cases. A single molecular test which targets additional genes could provide higher detection.

1. INTRODUCTION

In the year 2020, India reported 1.8 million cases of tuberculosis (TB), out of which 5.65 % were in children. TB meningitis is the deadliest form of tuberculosis which has disproportionately higher mortality than any other form of TB. The critical step in the management of TB meningitis is its early detection. Cerebrospinal fluid (CSF) biochemical and cytological parameters support the diagnosis of TB meningitis, but isolation of bacilli through nucleic acid amplification techniques or culture is of prime importance to determine the resistance pattern of the disease. WHO endorses the use of GeneXpert MTB/RIF for the diagnosis of pulmonary and extrapulmonary tuberculosis. GeneXpert targets an 81 bp core region of the rpoB gene known as Rifampicin Resistance Determining Region (RRDR). It has a Limit of Detection of 112.6 CFU per ml of sputum[1]. Second generation GeneXpert Ultra targets IS6110 and IS1081 along with four probes directed at Rifampicin Resistance Determining Regions of the rpoB gene. It has a limit of detection of 15.6 CFU/ml of sputum [1]. Various studies on GeneXpert has shown sensitivity of 72-88% [2-4] and specificity of >95% [2,3]

An Indian company, Molbio Diagnostics (BigTech India) has developed a technology for identification of M. tuberculosis complex (MTBC) in clinical samples and detection of rifampicin resistance, known as TRUENAT. TRUENAT is a portable mini RT-PCR device which has low cost, is battery operated, does not require air conditioning and is stable at temperatures upto 45C. TRUENAT MTB targets the nrdB gene which encodes for Ribonucleotide Diphosphate Reductase enzyme and it has a limit of detection of 100CFU/ml. An upgrade to this is TRUENAT MTB Plus which targets the nrdZ gene and IS6110 which has a limit of detection of 30CFU/ml. TRUENAT MTB RIF-DX targets the rpoB gene for detection of rifampicin resistance. TRUENAT has been evaluated for pulmonary samples and it shows sensitivity 58-88% [5-7] and specificity 72-98% [6-9] against culture. Only one study [10] has been conducted on CSF samples in adult patients with suspected TBM which shows sensitivity 78% and specificity 100% for TRUENAT MTB Plus. Currently, there is a paucity of literature on the use of TRUENAT to detect MTBC in the CSF of pediatric TBM patients, therefore this study was carried out to determine its role in the diagnosis of TBM and its concordance with GeneXpert in CSF.

2. MATERIALS AND METHODS

This was a cross-sectional study carried out in the Department of Pediatrics in a tertiary healthcare centre in North India after attaining ethics approval from Institutional Ethics Committee , Maulana Azad Medical College, New Delhi.

It included all strongly suggestive TBM patients as per NTEP guidelines [11] 2019 who were admitted and started on antituberculosis therapy (ATT) based on clinical, radiological and biochemical grounds.

2.1 Inclusion Criteria

Children < 18years who were strongly suggestive of TBM (NTEP) [1] as evidenced

by:Fever >5days, weight loss, anorexia along with neurological signs and symptoms (headache, vomiting, irritability, lethargy, seizures, confusion, coma, neck stiffness, cranial nerve palsy, hemiparesis) and CSF showing presence of 10-500 WBC/mm3, lymphocytes >50% and CSF/Plasma glucose <50% and WBC in blood <15x10^9/lt And/or imaging with evidence of basal meningeal enhancement, hydrocephalous or cerebral infarct or tuberculoma.

2.2 Exclusion Criteria

- Patients > 18years
- CSF not Suggestive of TB Meningitis (as per NTEP guidelines(1))

2.3 Methodology

75 cases strongly suggestive of TBM were enrolled in this study after taking due consent/assent. A detailed history, clinical examination, blood investigations and neuroimaging were carried out in the enrolled cases. CSF sample (3.5 ml) was obtained by standard technique. Of this, 1.5 ml was sent for cytology and biochemistry and 2 ml sample was processed for MTB- TRUENAT and GeneXpert as per the manufacturer's instructions.

Briefly, for TRUENAT, as per NTEP guidelines, the sample was first liquified to extract DNA and then mixed with the lysis buffer. Truenat AutoPrep V2 was used to extract and purify nucleic acids from the sample. It was then loaded into the PCR analyser. The result was obtained in the form of CT value and colony forming units CFU/ml for positive specimens. In case of positive results, TRUENAT MTB RIF test was used to determine rifampicin resistance.

For GeneXpert, the specimen was mixed with reagent in 1:2 dilution and incubated at room temperature for 15 minutes. The mixture was then transferred into the cartridge. The cartridge was loaded into the holder where the filtration, ultrasonic lysis, mixing with PCR reagent and amplification and detection occurred.

3. RESULTS

Our study included 75 cases of strongly suggestive TBM patients who presented to a tertiary care centre in New Delhi. Mean age of the patients was 10years and there were a few cases (2.7%) of infantile TBM. The youngest child affected was 5 months old. In our study, out of 75 cases, 46 (61%) were females. The male female ratio was 0.63. TB meningitis presents with fever- low grade, lasting for usually more than 5 days with some neurological involvement in the form of headache, altered sensorium, vomiting or seizures. CSF showed pleocytosis with lymphocyte predominance with cells count of more than 10/mm^3 in more than 90% cases. CSF had low glucose (85% had CSF glucose <50mg/dl) and high protein levels (90% had CSF protein > 100mg/dl).

Truenat detected MTB in 13 samples and GeneXpert detected in 16 samples. Out of these, in only 4 samples MTB was detected by both these tests. Four cases were found to be Rifampicin resistant- two by TRUENAT and another two by GeneXpert. Among these four cases, only one was MTB positive in both TRUENAT and GeneXpert, wherein TRUENAT detected Rifampicin resistance but GeneXpert could not detect resistance. Conflict resolution could not be done in this case because the patient expired before a repeat sample could be taken for the assay. GeneXpert showed indeterminate resistance in 2 cases.

To assess the degree of agreement, Cohens Kappa Coefficient was calculated which was 0.105 which depicted a slight agreement between TRUENAT and GeneXpert.

Concordance between TRUENAT and GeneXpert results: 72%.

7 out of 13 cases of TRUENAT positive had CSF protein between 100-200mg/dl. Higher protein (>200mg/dl) was associated with decreased positivity of TRUENAT, although this was not statistically significant. On the other hand, almost 50% of GeneXpert positive cases had CSF protein between 200 to 400mg/dl. High protein levels did not affect GeneXpert results.

Higher CSF count(>10cell) was associated with higher chances of positivity in GeneXpert as well as TRUENAT. However, there was no correlation with the percentage of lymphocytes in CSF with the positivity of GeneXpert and TRUENAT.

Table 1. Table showing clinical, radiological and CSF profiles

Characteristics	N (percentage)	Characteristics	N (percentage)
Age (years) mean	10.58 ± 6.11	STAGE I TBM	10 (13.3)
Males	29 (38.7)	STAGE II TBM	46 (61.3)
History of contact	29 (38.7)	STAGE III TBM	19 (25.3)
Symptoms		Tuberculin Skin Test Positive	12 (16)
Fever	75 (100)	HIV Positive	0(0)
Anorexia	57 (76)		
Weight loss	55 (73.3)	**Neuroimaging**	
Seizure	49 (65.3)	Hydrocephalous	46 (61.3)
Headache	38 (50.7)	Tuberculoma	14 (18.6)
Vomiting	33 (44)	Leptomeningeal enhancement	12 (16)
Altered sensorium	32 (42.7)	Basal exudates	10 (13.3)
Cough	20 (26.7)	Infarct	9 (12)
		Periventricular edema	9 (12)
Signs			
Neck rigidity	50 (66.7)		
Posturing	19 (25.3)	Chest Xray suggestive of pulmonary TB	15 (20)
Weakness	10 (13.3)		
6th Cranial nerve palsy	18 (24)		
7th cranial nerve palsy	4 (5.3)		
Kernig/ Brudzinski	7 (9.3)		
GCS<8 at presentation	18 (24)		
CSF Parameters			
CSF cells <10/mm^3	5 (6.7)	CSF protein 45-100 mg/dl	8(10.6)
CSF cells 10-100/mm^3	36 (48)	CSF protein 100-200 mg/dl	42(56)
CSF cells 100-500/mm^3	34 (45.3)	CSF protein 200-400 mg/dl	18(24)
CSF cells median (IQR)	75(37.5-150)	CSF protein >400 mg/dl	7(9.3)
		CSF protein median (IQR)	160 (123-238.5)
CSF lymphocytes > 80%	23 (30.7)		
CSF lymphocytes 50-80%	23 (30.7)	CSF glucose <50 mg/dl	64 (85.3)
CSF lymphocytes <50%	29 (38.7)	CSF glucose 50-100 mg/dl	11 (14.7)
CSF lymphocytes percentage median (IQR)	60 (40-90)	CSF Glucose median (IQR)	32 (22-40.5)

Table 2. Truenat and genexpert results in 75 tb meningitis patients <18years of age

	Truenat		Total
	Negative	Positive	
Genexpert negative count % of total	50	9	59
	66.7%	12.0%	78.7%
Positive count % of total	12	4	16
	16.0%	5.3%	21.3%
Total Count % of Total	62	13	75
	82.7%	17.3%	100.0%

4. DISCUSSION

Our study showed that although TRUENAT detected 13/75 cases (17.3%) and GeneXpert detected 16/75 cases (21.3%), there was only slight agreement between the results of these two tests because only 4 samples were positive on both the tests. GeneXpert was able to detect additional 12 cases and TRUENAT detected additional 9 cases of TBM. Higher CSF protein levels were not associated with TRUENAT or GeneXpert positive results.

Table 3. Shows concordance between TRUENAT and GeneXpert in various studies

S. No	STUDY	Comparison between	Samples	Patient	Concordance
1.	Our Study	TRUENAT vs GeneXpert	CSF	Pediatric	72%
2.	Nikam et al[11]	TRUENAT vs GeneXpert	Pulmonary	Adult	95.9%
3.	Gomathi et al[13]	TRUENAT vs GeneXpert	Pulmonary	Adult	79.9%
4.	Sharma et al[10]	GeneXpert ULTRA vs TRUENAT MTB Plus	CSF	Adult	68.5%
5.	Singh et al[6]	TRUENAT vs GeneXpert	Pulmonary	Pediatric	85.7%

Neither could we find any study establishing the relation between these parameters.

The difference in TRUENAT and GeneXpert results was also seen in a study from North India [5] on pulmonary samples of suspected TB patients. This study enrolled 612 patients out of which 111 were positive by TRUENAT MTB, but 55 out these 111 samples were negative by GeneXpert. Similarly, there were 32 cases which were positive by GeneXpert but not detected by TRUENAT. In this study, the sensitivity of TRUENAT against liquid culture was 58.3 % and that of GeneXpert was 53.6 %.

Another study by Sharma et al [10] on CSF samples in adult population was conducted in PGI Chandigarh. This study used TRUENAT MTB PLUS (TruPlus) and GeneXpert Ultra for detection of Mycobacterium tuberculosis in adult CSF samples. TruPlus has limit of detection of 30CFU/ml and GeneXpert Ultra has limit of detection of 15.6 CFU/ml. In this study, 108 suspect TBM patients were enrolled out of which 85 were positive on TruPlus and 73 were positive on Ultra but 23 out of 85 were not detected by Ultra and three among those were culture positive and 11 out of 73 were not detected by TruPlus.

But the first study conducted on TRUENAT by Nikam et al. [12] in 2014 with sputum samples had different results. Out of 247 samples, 229 samples i.e. 92.7% showed identical results for both Xpert and Truenat MTB. Out of the discordant results, 8 were positive by Xpert but negative by Truenat and 3 were negative by Xpert and positive by Truenat. Hence, this study showed higher concordance between GeneXpert and TRUENAT. The sensitivity of TRUENAT against MGIT as gold standard was 94.7%. Another study by Nikam et al. [13] showed 91% sensitivity of TRUENAT against clinically diagnosed pulmonary TB. But the data of this study might be influenced since the authors of this study were a part of the company involved in the manufacture of Truenat chips.

The discrepancy in the TRUENAT and GeneXpert results can be due to the following reasons:

a. TRUENAT is a multistep process in which liquefaction and lysis are done separately. This extraction process might help in decreasing the inhibitors of PCR present in the sample.
b. Proteins inhibit PCR studies in various ways. In our study, high protein levels were associated with decreased positivity in TRUENAT, although statistically not significant and on the other hand, high protein levels did not affect the GeneXpert results.
c. The two assays have different gene targets for the detection of MTBC. GeneXpert targets rpoB gene; while TRUENAT targets nrdB gene which may account for the different results in the two tests.
d. Moreover, the two tests use different technologies. GeneXpert uses molecular beacons, whereas TRUENAT uses taqman probes (Nikam et al. [13-14], 2013).

However, further studies need to be carried out to ascertain the cause of this discrepancy.

5. CONCLUSION

TBM diagnosis requires a combination of clinical, biochemical and radiological parameters as molecular tests alone cannot detect the bacilli in a large proportion of CSF of TBM patients. Isolation of Mycobacterium tuberculosis in pediatric population is difficult because of its paucibacillary nature and limited volume of CSF that can be collected for analysis. Although TRUENAT and GeneXpert are now used widely for the diagnosis of pulmonary and extrapulmonary TB, its utility in extrapulmonary samples needs to be evaluated further especially in pediatric population. Furthermore, as TRUENAT and GeneXpert look at different

genes, a single test which targets additional genes in both molecular tests could provide higher detection.

6. LIMITATIONS OF OUR STUDY

Since this study was based on a paediatric population, there was limited CSF sample that could be collected for analysis, especially in a sick child with raised intracranial tension, hence culture could not be carried out. So we were unable to calculate the sensitivity and specificity of TRUENAT for CSF samples.

7. STRENGTH OF OUR STUDY

As much as we could search the literature, this is the first study comparing the degree of agreement of TRUENAT and GeneXpert in suspected pediatric TBM patients.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT

Informed consent and/or assent from all parents and children, as applicable were obtained for inclusion in the study.

ETHICAL APPROVAL

Ethics approval or waiver has been obtained from Institutional Ethics Committee , Maulana Azad Medical College, New Delhi with their letter number F1/IEC/MAMC {82/10/2020/No 141} dated 14/01/2021.

REFERENCES

1. Chakravorty S, Simmons AM, Rowneki M, Parmar H, Cao Y, Ryan J. The new Xpert MTB/RIF Ultra: improving detection of Mycobacterium tuberculosis and resistance to rifampin in an assay suitable for pointof-care testing. MBio. 2017;8(4): e00812-17.

2. Metcalf T, Soria J, Montano SM, Ticona E, Evans CA, Huaroto L. Evaluation of the GeneXpert MTB/RIF in patients with presumptive tuberculous meningitis. PloS One. 2018;13(6):e0198695.

3. Patel VB, Theron G, Lenders L, Matinyena B, Connolly C, Singh R. Diagnostic accuracy of quantitative PCR (Xpert MTB/RIF) for tuberculous meningitis in a high burden setting: a prospective study. PLoS Med. 2013;10(10):e1001536.

4. Bahr NC, Nuwagira E, Evans EE, Cresswell FV, Bystrom PV, Byamukama A. Diagnostic accuracy of Xpert MTB/RIF Ultra for tuberculous meningitis in HIVinfected adults: a prospective cohort study. Lancet Infect. Dis. 2018;18(1):68-75.

5. Singh UB, Singh M, Sharma S, Mahajan N, Bala K, Srivastav A, Expedited diagnosis of pediatric tuberculosis using Truenat MTB-Rif Dx and GeneXpert MTB/RIF.Sci. Rep. 2023; 13(1):6976.

6. Meaza A, Tesfaye E, Mohamed Z, Zerihun B, Seid G, Eshetu K. Diagnostic accuracy of Truenat Tuberculosis and Rifampicin-Resistance assays in Addis Ababa, Ethiopia. Plos one. 2021;16(12):e0261 084.

7. Penn-Nicholson A, Gomathi SN, Ugarte-Gil C, Meaza A, Lavu E, Patel P. A prospective multicentre diagnostic accuracy study for the Truenat tuberculosis assays. Eur. Respir. J. 2021; 58(5).

8. Mangayarkarasi V, Sneka P, Sujith R. Jayaprakash, ergonomic diagnostic tool based on chip mini RT-PCR for diagnosis of pulmonary and extra pulmonary tuberculosis. J Pure Appl Microbiol. 2019; 13(2):1185-90.

9. Palacios CF, Saleeb PG. Challenges in the diagnosis of tuberculous meningitis. Journal of Clinical Tuberculosis and other Mycobacterial Diseases. 2020;20:100164.

10. Sharma K, Sharma M, Modi M, Singla N, Sharma A, Sharma N. Comparative analysis of truenat™ MTB plus and xpert® ultra in diagnosing tuberculous

meningitis. Int J Tuberc Lung Dis. 2021;25(8):626-31.

11. RNTCP Updated Pediatric TB Guidelines. Revised National Tuberculosis Control Program and Indian Academy of Pediatrics; 2019.

12. Nikam C, Kazi M, Nair C, Jaggannath M, Manoj M, Vinaya R. Evaluation of the Indian TrueNAT micro RT-PCR device with GeneXpert for case detection of pulmonary tuberculosis. Int J Mycobacteriol. 2014;3(3):205-10.

13. Nikam C, Jagannath M, Narayanan MM, Ramanabhiraman V, Kazi M, Shetty A et al. Rapid diagnosis of Mycobacterium tuberculosis with Truenat MTB: A near-care approach. PloS One. 2013;8 (1):e51121.

14. Gomathi NS, Singh M, Singh UB, Myneedu VP, Chauhan DS, Sarin R, et al. Multicentric validation of indigenous molecular test Truenat™ MTB for detection of Mycobacterium tuberculosis in sputum samples from presumptive pulmonary tuberculosis patients in comparison with reference standards. Indian J Med Res. 2020;152(4): 378.

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