EVALUATION OF TRUENAT WITH GeneXpert FOR DETECTION OF TUBERCULAR MENINGITIS IN CHILDREN AND ADOLESCENTS

1. ABSTRACT

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

Aim: To study the role of TRUENAT in diagnosis of 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 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. Concordance between the two tests was 72%. Cohen kappa (k-0.105) analysis showed slight agreement between the two tests.

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.

KEYWORDS – Tubercular Meningitis, Pediatrics, TRUENAT, GeneXpert, Cerebrospinal fluid

2. 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[2].

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. 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.

3. MATERIALS AND METHODS

This was a cross-sectional study carried out in the Department of Pediatrics in a tertiary healthcare centre in North India. It included all strongly suggestive TBM

patients as per NTEP guidelines[1] 2019 who were admitted and started on antituberculosis therapy (ATT) based on clinical, radiological and biochemical grounds.

3.1 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.

3.2 Exclusion criteria: None

3.3 Methodology: 75 cases strongly suggestive of TBM were enrolled in this study. 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.

4. 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).

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)		
6^{th} Cranial nerve palsy	18 (24)		
7^{th} 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 60 (40-90) median (IQR)	CSF Glucose median 32 (22-40.5) (IQR)

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. Rifampicin resistance was detected in 2 samples each in Truenat and GeneXpert. Only one among these four resistant cases was detected by both these tests. Conflict resolution could not be done in that case because the patient expired before a repeat sample could be taken for the assay. GeneXpert showed indeterminate resistance in 2 cases.

TABLE 2: TRUENAT and GeneXpert results in 75 TB meningitis patients <18years of age.

		TRUENAT		
		NEGATIVE	POSITIVE	Total
GeneXpert NEGATIVE	Count	50	9	59
	% of Total	66.7%	12.0%	78.7%
POSITIVE	Count	12	**4**	16
	% of Total	16.0%	5.3%	21.3%
Total	Count	62	13	75
	% of Total	82.7%	17.3%	100.0%

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.

5. 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.

Our study could not find any corelation of positivity of TRUENAT or GeneXpert in relation to CSF protein or cell count. We could not find any study establishing any relationship between these parameters.

The difference in TRUENAT and GeneXpert results was also seen in a study from North India[3] 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[4] 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.[5] 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.[6] 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[6], 2013).

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

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[5]	TRUENAT vs GeneXpert	Pulmonary	Adult	95.9%
3.	Gomathi et al[7]	TRUENAT vs GeneXpert	Pulmonary	Adult	79.9%
4.	Sharma et al[4]	GeneXpert ULTRA vs TRUENAT MTB Plus	CSF	Adult	68.5%
5.	Singh et al[3]	TRUENAT vs GeneXpert	Pulmonary	Pediatric	85.7%

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.

8. 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.

9. DECLARATIONS

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

10. EHICAL STATEMENT

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.

11. REFERENCES

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