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Original Article

TB LAMP assay, a beneficial tool for the diagnosis of Tubercular meningitis in resource-limited settings

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Abstract

Introduction: Tubercular meningitis (TBM) is a serious public health problem in developing countries as it leads to significant mortality and residual neurological sequelae. The estimated mortality due to TBM in India is 1.5 per 100,000 population. In resource-limited settings, only the Ziehl-Neelsen (ZN) stain, which has very little sensitivity, is available. The World Health Organization recommended the Loop Mediated Isothermal Amplification (TB LAMP) assay for pulmonary tuberculosis only. We evaluated this test for tubercular meningitis as well.

Methodology: In a cross-sectional study of 2-year duration, we have taken 239 cerebrospinal fluid samples from suspected cases of tubercular meningitis patients. ZN staining along with Mycobacteria Growth Indicator Tube (MGIT) TB culture, Xpert MTB/RIF Ultra assay, and commercial TB LAMP assay were performed for each sample.

Results: Out of 239 samples, 40 samples (16.73%) were found TB LAMP assay positive, 48 samples (20.08%) were found Xpert ultra-assay positive, 12 samples (5.02%) were MGIT TB culture positive and acid-fast bacillus smear positive in ten samples (4.18 %). Out of 12 MGIT-positive samples, all samples (100%) were TB LAMP and Xpert ultra positive and one sample (8.33%) was ZN smear positive. In 199 negative samples from the TB LAMP assay, eight samples were positive by Xpert, none by MGIT TB culture and AFB smear. Sensitivity and specificity were found as 100% and 87.66%, respectively, for the TB LAMP assay.

Conclusion: TB LAMP assay is a rapid, cost-effective, sensitive, and specific test for tubercular meningitis infection in resource-limited settings.

Key words: CSF; TB LAMP; MGIT; TBM; Xpert; ZN Smear.

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Introduction

Tubercular meningitis (TBM) is a grave public health problem in developing countries because it leads to significant mortality and residual neurological sequelae. The estimated mortality due to TBM in India is 1.5 per 100,000 population [1]. Although India is an endemic country for TBM, there is very little patient data available regarding clinical, radiological, and laboratory (biochemical and microbiological) parameters.

The clinical manifestations in many TBM patients are atypical. So, early diagnosis of TBM is crucial. TBM is diagnosed by smear microscopy, culture, or cartridge-based nucleic acid amplification test CBNAAT Xpert MTB/RIF assay. Smear microscopy has very low sensitivity, especially for extrapulmonary samples, and culture techniques are time-consuming. Xpert MTB/RIF assay, although costly, is available at various secondary care hospitals by the government of India, but it requires suitable infrastructure with a continuous power supply. In peripheral health centers, Xpert MTB/RIF is not feasible due to limited infrastructure and medical resources. We may replace Xpert MTB/RIF with Loop Mediated Isothermal Amplification (TB LAMP) in these peripheral centers.

The National Strategic Plan (2012-2017) of the government of India, under which the National Tuberculosis Elimination Program (NTEP) comes, identified certain areas in the diagnosis and treatment of TBM, out of which one is to deploy improve rapid diagnosis at the field level "and one of them is to improve rapid diagnosis at the field level [2]. Thus, LAMP assay could be a potentially valuable tool for the rapid diagnosis of tuberculosis in the field setup where there is no expertise available, as well as no infrastructure present, for carrying out sophisticated molecular methods. The World Health Organization (WHO) has recommended the commercial TB-LAMP assay as a replacement or follow-up test for sputum smear microscopy for pulmonary tuberculosis diagnosis [3] but various studies reported the use of LAMP assay for the detection of extrapulmonary

tuberculosis. *Mycobacterium tuberculosis* is detected by acid-fast bacillus (AFB) staining, culture, and molecular testing for diagnosis of TBM. TB-LAMP testing for rapid diagnosis of TBM is a new approach. There are hardly a few studies that confirmed the utility of TB LAMP in the diagnosis of TBM in India [4-5] and not a single study was found from Uttar Pradesh for the use of TB-LAMP in the diagnosis of TBM.

Methodology

Ethical statement

The Institutional Ethical Committee approved this study (Approval letter IEC No. 13/18, dated 08/10/2018). Patients with suspected CNS TB infection who were willing to give informed written consent to participate in the study were taken for evaluation.

Type of study and duration

This is an analytical cross-sectional study that lasted for 2 years (2020-2022).

Sample size

We have taken 239 cerebrospinal fluid (CSF) samples from all clinically suspected TBM patients by a simple random sampling.

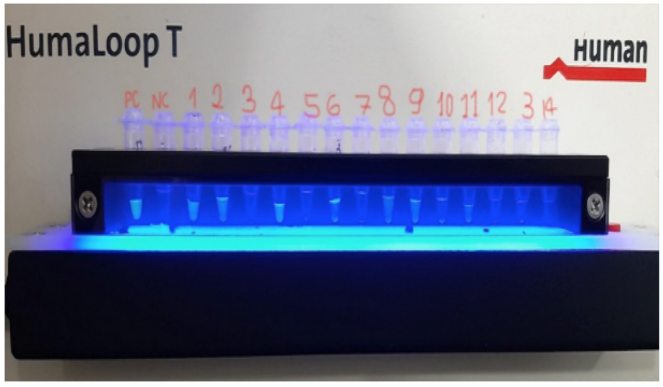
Inclusion criteria

A patient with suspected TBM was included in the study according to WHO-defined criteria of definite, probable, and possible TBM groups based on clinical criteria, CSF criteria, cerebral imaging criteria, and evidence of tuberculosis elsewhere [6].

Exclusion criteria

Patients with any microbiological evidence of other CNS infections were excluded from the study.

Figure 1. Interpretation of TB LAMP assay.



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Specimen collection

CSF (1-3 mL) was collected by lumber puncture and collected into a sterile screw-cap tube. The sample was transported to a microbiology laboratory within one hour. The sample was processed immediately in the laboratory or it was stored at -20 °C.

Specimen processing

CSF was centrifuged and the sediment was used for processing different types of microbiological testing.

Samples were divided into three parts. The first part was used for smear and culture by MGIT-960 (BD BACTEC MGIT™). The second part was used for performing Xpert Ultra MTB/RIF assay (Cepheid, India) as per the user's manual. The third part was analyzed by the commercial TB LAMP assay (Loopamp™ PURE & MTBC-Eiken Chemical Ltd.)

TB LAMP assay procedure

1. Sample preparation (10 -20 minutes):
   a. Using a wide-bore disposable pipette (Eiken Chemical Co., Ltd.), 120 µL of each CSF sample were transferred to a heating tube containing the extraction solution;
   b. Samples were inverted 3-4 times and placed in the heating block at 90 °C for 5 minutes to lyse and inactivate mycobacteria;
   c. Tubes were removed from the heating block and let cool down for 2 minutes;
   d. Heating tubes were attached to an adsorbent tube and mixed by shaking until all the powder has completely mixed with the solution;
   e. An injection cap was placed on top of each adsorbent tube and screwed tightly to pierce the seal;
   f. The nozzle was inserted in the reaction tube and a few drops of the solution (30 µL) were transferred to the reaction tube.
2. Amplification (40 minutes):
   a. The sample incubator was set at 67 °C;
   b. Reaction tubes were placed into the heating block to start the reaction;
   c. The amplification was stopped after 40 minutes.
3. Visual detection of fluorescence light from the reaction tube using UV light (0.5 -1 minute):
   a. The reaction tubes were transferred to the fluorescence detector and results recorded (Figure 1);
   b. Hazardous waste (reaction tubes) was burned using an incinerator. Negative and positive

controls (present in the kit) with test samples were run each time.

Statistical analysis

Data analysis was conducted using Statistical Package for the Social Sciences software (SPSS version 21.0, IBM Corporation, US) and Microsoft Office Excel 2010 for discrete variables, which were represented in frequency and proportions. Additionally, 95% confidence intervals were calculated for the estimated proportions of study variables. The chi-square test was employed to measure the association between study variables. A p value less than 0.05% was considered significant. Test diagnostic criteria were assessed using sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratio, and kappa statistics for inter-rated reliability for qualitative study variables.

Results

Diagnosis of TBM

Of 239 patients with suspected TBM, 12 patients were classified as 'definite cases' of TBM (culture positive), whereas 76 patients as 'probable' (culture negative) and 151 patients as 'possible cases'(culture negative) of TBM. All 'definite cases' (12/12) were found TB LAMP assay positive (100%) and 28/76 'probable cases' were TB LAMP positive (37.3%) but none of the'possible cases' (0/151) was TB LAMP assay positive (Table 1).

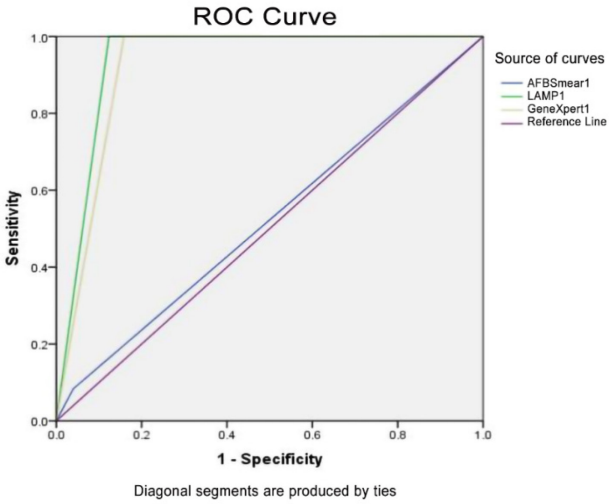
Demographic

In this study, 40 CSF samples were diagnosed as TBM positive by TB LAMP assay. Out of those, 22 were males and 18 females with a mean age of 46.15 ± 25.82.

Clinical picture

Among the clinical symptoms, the majority were statistically significant. TBM was confirmed by examination of CSF from 52 patients (22%), out of which 12 were in the 'definite case' group, 34 were in the 'probable case' group, and six were in the 'possible' group. The radiological analysis detected TBM in 47 patients (20%), out of which 10 patients were in the 'definite case' group, 32 patients in the 'probable' and seven in the 'possible group'. History of pulmonary TB was found in 40 patients and contact history was present in 99 patients. HIV was detected in one patient who was not found with TBM by any of the tests.

Figure 2. ROC curve analysis of the sensitivities and specificities of the TB LAMP methods.



Sensitivity and specificity of TB LAMP assay

In the 239 patients with suspected TBM, AFB staining (ZN staining), MGIT960 TB culture, Xpert MTB/RIF ultra-assay, and TB LAMP assay were performed. The sensitivity of AFB staining, Xpert MTB/RIF ultra-assay, and TB LAMP assay was 8.3%, 100%, and 100%, respectively. Specificity was 96%, 84.14%, and 87.66%, respectively. The positive likelihood ratio was higher for the TB LAMP assay. Twelve samples showed concordance between the results of the culture-based method and the LAMP assay. None of the samples was culture positive or LAMP negative. Twenty-eight samples were culture negative and LAMP positive. TB LAMP assay showed very good detection accuracy (a larger value on the y-axis [TPR] indicated higher diagnostic accuracy), as compared to the culture method (gold standard), with a significant difference ($p < 0.001$). The ROC curve analysis revealed significant diagnostic accuracy of TB LAMP assay with 100% sensitivity and 87.66% specificity (Figure 2). TB LAMP assay showed moderate agreement with a kappa value.

Table 1. Distribution of suspected TBM cases according to 2010 consensus criteria.

Classification	Total (n = 239)	LAMP Positive (n = 40)
Definite (Culture +)	12 (0.5%)	12
Probable (Culture -)	76 (32%)	28
Possible (Culture -)	151 (63%)	0

Discussion

TBM is a serious public health problem in developing countries, as it leads to significant mortality and residual neurological sequelae. Due to the nonspecific clinical presentation and unavailability of detection methods, diagnosis of TBM is very challenging, especially in peripheral health settings.

The most significant advantage of the TB LAMP is that the entire assay takes about one hour, saving a significant amount of time and it can be performed under isothermal conditions (60 -65 °C) eliminating the need for specialized equipment or expertise.

Evaluation of TB LAMP assay in suspected cases of TBM

Notomi *et al.* first developed the LAMP assay in 2000 [8]. LAMP is a nucleic acid amplification technique (NAAT) that is well suited for operating in the laboratory with minimum infrastructure; it is a likely point-of-care diagnostic test for TB. This test does not require any infrastructure like an air-conditioning room or any major equipment, like a thermocycler, as amplification is performed at a constant temperature, which does not require a thermal cycler and detector, the whole amplification can be done within 60 minutes. It is performed using four types of primers with strand displacement activity based on six distinct regions of the reaction carried out at a constant temperature. It has high amplification efficiency, amplifying DNA 109-1010 times in 15-60 minutes. Amplification and gene detection can be accomplished in a single step.

WHO in 2016 endorsed the use of commercial LAMP assay for diagnosis of pulmonary tuberculosis in peripheral health settings. In this study, an attempt has been made to evaluate this commercial TB LAMP assay for diagnosis of TBM. We evaluated that very little volume of the sample was required, in comparison with other molecular methods. For other tests, a minimum of 500 µL to 1 mL of CSF is required, but for this test, a minimum of 100 µL amount of CSF was sufficient. In extra-pulmonary samples like CSF, amplification inhibitors can cause hindrance in a test, but in this case, this problem was not present. The presence of fluorescence indicated the presence of the target gene, and visual detection was achieved.

Most of the cases of TBM were reported in rural areas and among individuals with low socioeconomic status, as shown in previous studies cited in this study. The timely diagnosis was very difficult for them at primary and even secondary care centers. As a result, they were often referred to the nearest tertiary care center. However, due to delays in management, many patients experienced severe morbidity and mortality (Table 2).

Comparison of TB LAMP assay with smear microscopy, MGIT culture, and Xpert MTB/RIF Ultra assay

In our study, we have tried to compare commercial LAMP assay with other diagnostic tests like conventional AFB staining and molecular Xpert MTB/RIF ultra-assay with MGIT culture taken as the gold standard. Our study demonstrated high sensitivity (100%) for LAMP assay that is equal to Xpert MTB/RIF ultra-assay as compared to AFB staining (8.3%), but the specificity of AFB staining is little higher: 96.04% as compared to TB LAMP assay (specificity 87.67%). Nagadev *et al* [4] from south India reported sensitivity of LAMP assay at 88% and specificity at 80% in their study using in-house LAMP assay for diagnosis of TBM. After that, Modi *et al.* from north India did in house LAMP test on two separate target genes in patients with TBM and reported a sensitivity of 83% and specificity of 100% by using the IS6110 targeted gene and 87% sensitivity and 100% specificity by using MPB64 as a targeted gene [5]. Our study had higher sensitivity because our study used a WHO-approved commercial TB LAMP kit compared

Table 2. Demographic analysis.

		TB LAMP Assay	
		Positive (n = 40)	**Negative (n = 199)**
Demography	Urban	18	181
	Rural	22	177
Gender	Male	22	177
	Female	18	181
Age (years)	0-10	2	6
	11-20	11	27
	21-30	12	41
	31-40	2	29
	41-50	4	23
	50-60	5	29
	> 60	4	44

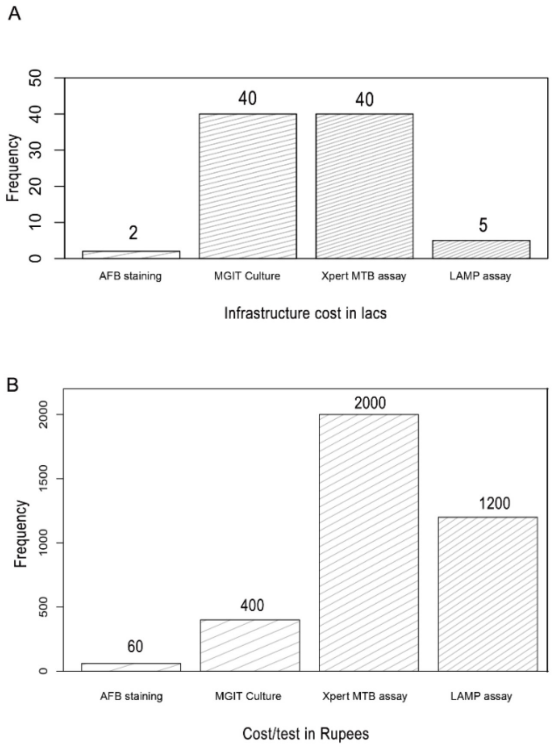
to using an in-house TB LAMP kit by others that might have some technical limitations (i.e., insufficient CSF volume, partial lysis of cells, loss of DNA during purification).

We have evaluated this LAMP assay with Xpert MTB/RIF Ultra assay. Xpert MTB/RIF Ultra assay is a modified version of Xpert MTB/RIF assay that has high sensitivity. Interpretation of the test is in the form of very high, high, low, and trace MTB detected. In our study, all 48 Xpert ultra-positive samples were interpreted as very high (eight samples), high (ten samples), low (18 samples), and 12 sample trace MTB detected by Xpert ultra-MTB assay. Those samples detected as a trace in the early part of the study (eight samples) were not found positive by LAMP assay but in the later part of the study after slight technical modifications (rpm and duration of centrifugation increased) trace MTB detected samples (four samples) were also found positive by LAMP assay. In conclusion, out of 48 positive samples by Xpert MTB/RIF ultra-assay, only eight samples were negative by TB LAMP assay.

Cost analysis and affordability

This test is economical after evaluating the infrastructure and cost per test compared to the Xpert MTB/RIF assay, which was endorsed by WHO for extrapulmonary samples (Figure 3).

Figure 3. Cost and affordability analysis of different tests for the diagnosis of TBM. A: Infrastructure evaluation of different tests; B: Cost per test evaluation of different tests.



In our study sensitivity of both tests was equal, but cost analysis showed that the LAMP assay was cost-effective. The cost of Xpert equipment was significantly higher than that of TB-LAMP, and the test kit cost was also higher for Xpert compared to TB-LAMP kits. Therefore, if we use Xpert at primary and secondary care centers, it will put a significant burden on the national tuberculosis elimination program budget in India as a developing country. A study from Vietnam and Malawi by Sohn *et al* in 2019 also provided a comprehensive assessment of the cost and affordability of TB-LAMP compared to Xpert as a potential alternative upfront peripheral NAAT in high TB burden countries [9]. The advantage of rifampicin resistance detection by Xpert MTB/RIF over LAMP assay may limit the cost-utility of LAMP. However, drug resistance in extrapulmonary TB is uncommon to date, and timely detection of TBM is far more important for early management at peripheral health centers.

Conclusions

LAMP assay was found to be rapid, sensitive, more economical, and beneficial for detecting TBM as compared to other tests. It can replace microscopy at primary and secondary health care centers, enabling early diagnosis of tubercular meningitis and reducing mortality and morbidity.

Limitations of the study

This study had a small sample size due to cost constraints. For a proper evaluation of the assay, a large sample size is required.

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