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Original Article

Microbiology Section

Comparative Evaluation of Various Microbiological Diagnostic Methods for Tubercular Meningitis: A Cross-sectional Study From a Tertiary Care Hospital

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ABSTRACT

Introduction: Tubercular Meningitis (TBM) is a major public health problem that results from the dissemination of *Mycobacterium tuberculosis* (MTB) to the Cerebrospinal fluid (CSF) and meninges. Despite the availability of effective treatment options, the mortality rate remains high, primarily due to diagnostic uncertainty or delay.

Aim: To compare Ziehl-Neelsen (ZN) staining, liquid culture using the *Mycobacterium* Growth Indicator Tube (MGIT 960) system, and Cartridge-Based Nucleic Acid Amplification Test (CB-NAAT) in the diagnosis of TBM.

Materials and Methods: This cross-sectional study was conducted in the Department of Microbiology, ABVIMS and Dr. RML Hospital, New Delhi, India from 1st June 2020 to 28th February 2021. A total of 400 CSF samples from patients with clinical suspicion of TBM were included. Each sample was divided into three parts for ZN staining microscopy, CB-NAAT, and liquid culture using MGIT 960. Demographic details such as age and gender were recorded in a prescribed proforma. Validity parameters including sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) were evaluated using Epi Info version 7.2. A p-value of <0.05 was considered statistically significant.

Results: Among the 400 clinically suspected patients, 48 (12%) were diagnosed positive for TBM. The majority of positive cases were observed in the 21-40 years age group, with an almost equal male-to-female ratio. The sensitivities of ZN staining, liquid culture, and CB-NAAT were 20.83%, 68.75%, and 76.19%, respectively. The specificity was 100% for all three diagnostic modalities. Among the 33 CB-NAAT-positive cases, eight patients were found to be rifampicin-resistant.

Conclusion: For early detection of *Mycobacterium tuberculosis*, CB-NAAT serves as a game-changer, as it provides results within two hours and simultaneously detects rifampicin resistance. Although liquid culture (MGIT) remains the gold standard for *Mycobaterium tuberculosis* detection, it requires approximately four to six weeks to yield results.

Keywords: Cartridge based nucleic acid amplification test, Cerebrospinal fluid, *Mycobacterium* growth indicator tube, *Mycobacterium tuberculosis*, Ziehl-neelsen

INTRODUCTION

According to the World Health Organisation (WHO) 2018 report on tuberculosis, 10 million people were diagnosed with the disease, and 1.5 million died from it [1]. Among all forms of extrapulmonary tuberculosis, Tubercular Meningitis (TBM) is the most fatal. It is a serious public health problem in India and constitutes approximately 1% of all tuberculosis cases, as per estimates for extrapulmonary tuberculosis by the Indian National Guidelines [2]. A large number of cases in India remain unnotified, undiagnosed, or inadequately diagnosed and treated in the private sector [3]. The microbiological diagnosis of TBM is particularly challenging due to the low bacillary load in CSF samples. Even today, no single diagnostic test can detect the infection with 100% sensitivity and accuracy. The nucleic acid amplification test, CB-NAAT (GeneXpert MTB/RIF assay), was recommended by the WHO in 2013 for the diagnosis of tuberculosis in children and extrapulmonary specimens. It was later recommended as an initial diagnostic test for paucibacillary disease, in preference to conventional microscopy and culture methods [4,5]. Despite these advancements, diagnostic uncertainty persists, often leading to delayed diagnosis and increased mortality. By reducing the turnaround time for diagnosis, mortality rates can be significantly decreased. Currently, diagnosis still relies on multiple tests, as well as clinical and imaging findings. No prior data or studies have been available regarding the best diagnostic method for TBM in our institute.

Considering this, present study aimed to compare different diagnostic modalities for TBM by evaluating their sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV). The role of CB-NAAT in the early detection of tuberculosis, along with its diagnostic accuracy compared to liquid culture using MGIT, was also assessed. This study may assist clinicians in achieving an early diagnosis of TBM and determining rifampicin sensitivity, thereby facilitating the initiation of appropriate treatment.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Microbiology, Atal Bihari Vajpayee Institute of Medical Sciences and Dr. Ram Manohar Lohia Hospital, New Delhi, India. A total of 400 CSF samples received from clinically suspected cases of TBM between 1st June 2020 and 28th February 2021 were included in the study. Since only routine diagnostic samples were analysed, ethical approval was not required.

Inclusion criteria: Samples from patients with a clinical suspicion of TBM were included in the study.

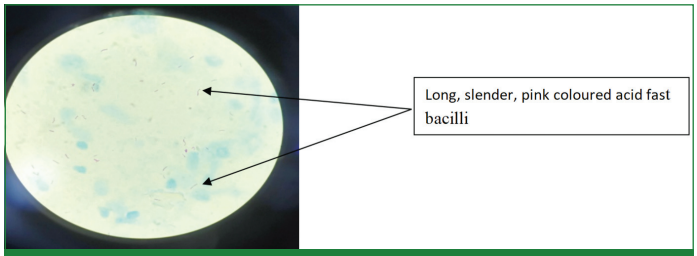
Exclusion criteria: Patients already receiving Antitubercular Therapy (ATT), as indicated on the requisition slip, samples with incomplete clinical history or missing details on the requisition form were excluded from the study.

Study Procedure

CSF samples from suspected cases of TBM received in the Microbiology Department were centrifuged at 3,000-3,500 × g for 15-20 minutes. The sediments were then used for smear preparation, MGIT inoculation, and the CB-NAAT test.

Acid-Fast Bacilli (AFB) staining and microscopy were performed according to the Revised National Tuberculosis Control Programme (RNTCP) guidelines [5]. One drop of the centrifuged CSF deposit was placed in the center of a clean glass slide and allowed to air dry. The smear was heat-fixed and stained with 1% hot carbol fuchsin. After five minutes, the stain was rinsed off with distilled water, followed by decolorisation using 3% acid alcohol for 2-3 minutes. The slide was again washed with distilled water and counterstained with 0.1% methylene blue for one minute. Finally, the stain was washed off with distilled water and allowed to air dry.

Mycobacterium tuberculosis appears as long, slender, beaded, red-colored bacilli under the microscope [Table/Fig-1].



[Table/Fig-1]: Microscopic view of *Mycobacterium tuberculosis* under 100x magnification.

For liquid culture, 500 µL of CSF sample was added to each MGIT tube, which already contained 800 µL of the antibiotic mixture (PANTA: polymyxin-B, amphotericin-B, nalidixic acid, trimethoprim, and azlocillin) and a growth supplement solution. All inoculated MGIT tubes were loaded into the automated MGIT-960 system in the slots assigned by the instrument after barcode scanning.

All tubes flagged positive were further processed for Ziehl-Neelsen (ZN) staining, while all tubes flagged negative were removed after the completion of 42 days of incubation. The MPT64 antigen card test was performed on all smear-positive MGIT tubes as per the manufacturer's instructions [6]. The CB-NAAT test was conducted according to the manufacturer's protocol [7].

STATISTICAL ANALYSIS

Data were entered into a Microsoft Excel spreadsheet. Categorical variables were summarised as frequencies and percentages, while continuous variables were presented as mean±standard deviation. Data analysis was performed using EpiInfo version 7.0. To evaluate validity parameters such as sensitivity, specificity, PPV, and NPV, EpiInfo version 7.2 was used. Diagnostic accuracy was expressed as percentages along with their 95% confidence intervals (CI).

RESULTS

During the study period, 400 CSF samples received from clinically suspected TBM patients were included. Among these, 48 (12%) were finally diagnosed as TBM. The most common age group affected was 21-40 years, with a median age of 35 years [Table/Fig-2]. Of the 400 samples, 216 were from females and 184 from males, giving a male-to-female ratio of 1:1.17. The most common clinical feature among these TBM cases was fever, followed by weight loss and loss of appetite.

S. No.	Age (years)	n (%)
1.	<20	6 (13)
2.	21-40	21 (44)
3.	41-60	16 (33)
4.	61-70	2 (04)
5.	>70	3 (06)

[Table/Fig-2]: Age distribution of positive cases of TBM.

Ten (2.5%) samples were positive by ZN staining, with a sensitivity of 20.83% and specificity of 100%. The PPV and NPV were 100% and 90.26%, respectively [Table/Fig-3].

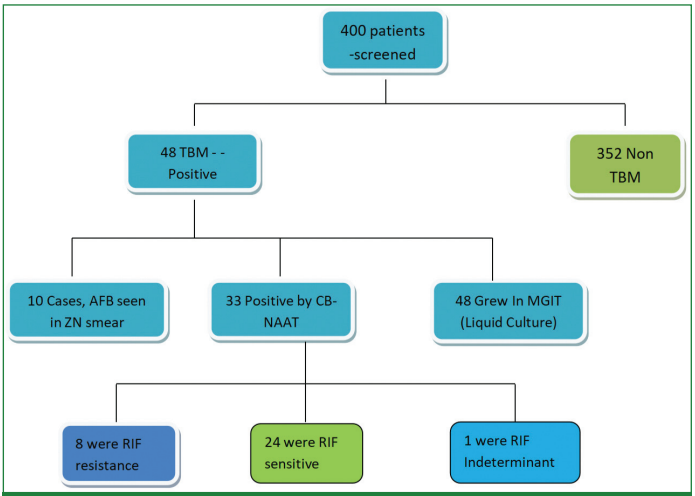
S. No.	Parameter	Estimate	95% confidence intervals
1.	Sensitivity	20.83	10.47-34.99
2.	Specificity	100	98.96-100
3.	Positive Predictive Value (PPV)	100	89-100
4.	Negative Predictive Value (NPV)	90.26	88.90-91.46

[Table/Fig-3]: Diagnostic evaluation of ZN Microscopy for TBM.

CB-NAAT detected MTB in 33 (8.25%) CSF samples, with a sensitivity of 68.75%, specificity of 100%, PPV of 100%, and NPV of 95.91% [Table/Fig-4]. CB-NAAT detected 23 (5.75%) additional MTB-positive cases that were AFB smear-negative. None of the samples showed a high bacterial load in CB-NAAT. Rifampicin resistance was detected in eight cases, and one case was found to be rifampicin indeterminate [Table/Fig-5].

S. No.	Parameter	Estimate	95% confidence intervals
1.	Sensitivity	68.75	53.75-81.34
2.	Specificity	100	98.96-100
3.	Positive Predictive Value (PPV)	100	97-100
4.	Negative Predictive Value (NPV)	95.91	93.89-97.28

[Table/Fig-4]: Diagnostic evaluation of CBNAAT for TBM.



[Table/Fig-5]: Flow chart of results.

When evaluated against CB-NAAT, liquid culture detected 48 (12%) MTB-positive CSF samples in MGIT media, showing sensitivity 76.19%, specificity 100%, PPV 100%, and NPV 95.91% [Table/Fig-6]. Liquid culture identified 15 (3.75%) additional positives that were CB-NAAT negative, and 38 (9.5%) additional positives compared to AFB microscopy. Only 10 (2.5%) samples were positive by all three diagnostic methods.

S. No.	Parameter	Estimate	95% confidence intervals
1.	Sensitivity	76.19	63.79-86.02
2.	Specificity	100	98.96-100
3.	Positive Predictive Value (PPV)	100	99-100
4.	Negative Predictive Value (NPV)	95.91	93.78-97.33

[Table/Fig-6]: Diagnostic evaluation of liquid culture for TBM.

DISCUSSION

Diagnosis of TBM remains highly challenging due to its non specific clinical presentation, poor sensitivity of smear microscopy, and the slow growth rate of *Mycobacterium tuberculosis*. Although multiple

diagnostic tests are currently available, no single test is sufficient for definitive diagnosis.

Present study included 400 CSF samples from patients with clinical suspicion of TBM. Of these, 216 were female and 184 were male, giving a male-to-female ratio of 1:1.17. The most commonly affected age group was 21-40 years, with a median age of 35 years, findings consistent with the study by Kamboj P et al., [8] from AIIMS Rishikesh. Other studies from India have also reported a higher incidence of TBM in young adults aged 18-40 years [9,10].

ZN smear microscopy is the oldest method for detecting *M. tuberculosis* bacilli and requires at-least 5,000-10,000 bacilli per mL of sample for reliable detection [11]. However, its results depend on sample volume, time to transport, and technical expertise of labouratory personnel. Present study found a sensitivity of 20.83%, comparable to results reported by Kent SJ et al., and Marais S et al., [12,13]. Due to poor sensitivity and low bacterial load in CSF, many TBM cases remain undiagnosed. Previous reports suggest that the diagnostic yield of microscopy can be improved by increasing the CSF sample volume and spending more time examining the smear [14-16].

Mycobacterial culture is considered the gold standard for confirming the diagnosis of tuberculosis. It requires only 100 bacilli per mL of sample, making it more sensitive than smear microscopy [11]. Present study, found a sensitivity of 76.19% using the liquid culture method, which was in concordance with a similar study conducted by Iqbal I et al., [17]. Hence, liquid culture provides good sensitivity; however, due to the slow-growing nature of *Mycobacterium tuberculosis*, it is a time-consuming process, making it less practical for clinicians when making treatment initiation decisions.

The overall sensitivity of CB-NAAT in present study was 68.75%, with 100% specificity, 100% PPV, and 95.91% NPV. Similar sensitivities were reported in studies by Nhu NT et al., Wang T et al., and Bohr NC et al., who observed sensitivities of 59%, 62%, and 60%, respectively [18-20]. Although CB-NAAT can be used to confirm TBM, it cannot be used to rule it out because of the possibility of false-negative results. Therefore, in cases of negative CB-NAAT results, further diagnostic tests must be performed to accurately exclude TBM.

Present study found eight cases of rifampicin resistance detected by CB-NAAT. In comparison, Iqbal I et al., reported 2% rifampicin-resistant cases, while Nhu NT et al., found four patients with rifampicin resistance [11,12]. As rifampicin resistance tends to develop later than isoniazid resistance, detection of rifampicin resistance by CB-NAAT indicates multidrug-resistant (MDR) tuberculosis. Therefore, for early definitive diagnosis, especially in MDR-endemic regions, CB-NAAT is a valuable diagnostic tool for detecting TBM. However, it should not be used as a stand-alone test to rule out TBM.

Liquid culture remains superior to all other diagnostic techniques, as it is 100% confirmatory, useful for anti-TB drug resistance detection, and allows effective monitoring of treatment response-including treatment success, failure, or relapse. The only major disadvantage is that it is time-consuming, which contributes to diagnostic delay.

Limitation(s)

The main limitation of present study was that, being a single tertiary care centre-based study, the results cannot be generalised to the entire population. Secondly, clinical follow-up and a reference standard for sample analysis by CB-NAAT and culture were not available due to a lack of regular patient monitoring.

CONCLUSION(S)

Present study clearly demonstrates the superiority of liquid culture (MGIT) over CB-NAAT in terms of sensitivity. However, it should be noted that CB-NAAT offers a much shorter turnaround time. Therefore, CB-NAAT can be used as an initial diagnostic test for early diagnosis of TBM, but it cannot be relied upon to rule out the disease. Hence, microbiological diagnosis must always be assessed in conjunction with other parameters such as clinical features, radiological findings, and CSF cell counts, among others.

Authors' contributions: AS: Conceptualisation of the study, research design, data analysis, and interpretation, and drafted and revised the manuscript. RS: Contributed to manuscript preparation, editing, data analysis, and interpretation. AS: Responsible for performing the tests and collating the data. SK: Contributed to study conceptualisation, supervised the research design, and critically reviewed the manuscript. All authors read and approved the final manuscript.

REFERENCES

[1] WHO Tuberculosis report September 2018. Available from: https://www.who.int/news-room/fact-sheets/detail/tuberculosis.

[2] Sharma SK, Ryan H, Khaparde S, Sachdeva KS, Singh AD, Mohan A, et al. Index-TB guidelines: Guidelines on extrapulmonary tuberculosis or India. Indian J Med Res. 2017;145:448-63.

[3] National strategic plan for tuberculosis elimination. 2017-2025. NSP; 2017.

[4] WHO. World Health Organization. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary and extra pulmonary TB in adults and children: policy update 2013; https://apps.who.int/iris/handle/10665/112472.

[5] RNTCP. Revised National Tuberculosis Control Programme. Standard operating procedures for Mycobacteriology laboratory. ICMR 2010; http://nirt.res.in/pdf/bact.SOP.pdf.

[6] Martin A, Bombeeck D, Mulders W, Fissette K, De Rijk P, Palomino JC. Evaluation of the TB Ag MPT64 Rapid test for the identification of Mycobacterium tuberculosis complex. Int J Tuberc Lung Dis. 2011;15:703-05.

[7] Xpert MTB/RIF kit inserts. http://www.cepheid.com/manageddownloads/xpert-mtb-rif-english-package-insert-301-1404-rev-b-february-2015.pdf.

[8] Kamboj P, Mathuria YP, Chakraborty D, Gupta P, Anand P. CBNAAT as a tool for revolutionizing the Diagnosis of Tuberculous Meningitis in Adults: Insights from a Tertiary Care Hospital in Northern India. IJSDR. 2023;8(8):68-73.

[9] Kaur H, Sharma K, Modi M, Sharma A, Rana S, Khandelwal N, et al. Prospective analysis of 55 cases of Tuberculosis Meningitis (TBM) in North India. J Clin Diagn Res. 2015;9(1):DC15- DC19.

[10] Modi M, Sharma K, Prabhakar S, Goyal MK, Takkar A, Sharma N, et al. Clinical and radiological predictors of outcome in tubercular meningitis: A prospective study of 209 patients. ClinNeurolNeurosurg. 2017;161:29-34.

[11] Diagnostic standards and classification of tuberculosis in adults and children, American Thoracic Society. Am J Respir Crit Care Med. 2000;161(4):1376-95.

[12] Kent SJ, Crowe SM, Yung A, Lucas CR, Mijch AM. Tuberculosis meningitis: A 30 year review. Clin Infect Dis. 1993;17(6):987-94.

[13] Marais S, Thwaites G, Schoeman JF, Torok ME, Misra UK, Prasad K, et al. Tuberculous meningitis: A uniform case definition for use in clinical research. Lancet Infect Dis. 2010;10(11):803-12.

[14] Heemskerk AD, Donovan J, Thu DDA, Marais S, Chaidir L, Dung VTM, et al. Improving the microbiological diagnosis of tuberculous meningitis: A prospective, international, multicentre comparison of conventional and modified Ziehl-Neelsen stain, GeneXpert, and culture of cerebrospinal fluid. J Infect. 2018;77(6):509-15.

[15] Wang YY, Xie B di. Progress on diagnosis of tuberculous meningitis. Methods Mol Biol. 2018;1754:375-86.

[16] Thwaites GE, Chau TTH, Farrar JJ. Improving the bacteriological diagnosis of tuberculous meningitis. J Clin Microbiol. 2004;42:378-79.

[17] Iqbal I, Farhana A, Zahoor D, Bashir H, Detection of tuberculous meningitis by various microbiological modalities at a tertiary care hospital in North India. Indian J Microbial Res. 2020;7(3):273-28.

[18] Nhu NT, Heemskerk D, Thu do DA, Chau TT, Mai NT, Nghia HD, et al. Evaluation of GeneXpert MTB/RIF for diagnosis of tuberculous meningitis. J Clin Microbiol. 2014;52(1):226-33.

[19] Wang T, Feng GD, Pang Y, Liu JY, Zhou Y, Yang YN. High rate of drug resistance among tuberculous meningitis cases in Shaanxi province, China. Sci Rep. 2016;6:25251.

[20] Bahr NC, Tugume L, Rajasingham R, Kiggundu R, Williams DA, Morawski B. Improved diagnostic sensitivity for tuberculous meningitis with Xpert MTB/RIF of centrifuged CSF. Int J Tuberc Lung Dis. 2015;19(10):1209-15.

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