CBNAAT as a tool for revolutionizing the Diagnosis of Tuberculous Meningitis in Adults: Insights from a Tertiary Care Hospital in Northern India

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Abstract-
Introduction: Tuberculous meningitis (TBM) remains a challenging and potentially fatal form of extrapulmonary tuberculosis. The conventional methods for TBM diagnosis, such as microscopy and culture, have limitations in terms of sensitivity and speed. The Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) offers a rapid and reliable diagnostic approach for Mycobacterium tuberculosis detection and rifampicin resistance. This study aimed to evaluate the diagnostic usefulness of CBNAAT in diagnosing TBM in adults at a tertiary care hospital in Northern India.
Methodology: This study was carried out at the Department of Microbiology, AIIMS, Rishikesh from January 2019 to March 2022. A total of 132 patients admitted with suspicion of tuberculous meningitis in our hospital during the study period were included in this study. Cerebrospinal fluid (CSF) samples were collected from patients and subjected to tests for Zeihl-Neelsen (ZN) staining, CBNAAT and culture.
Results: CBNAAT identified MTB in 63/128 samples from the patients (49.2%). In relation to composite reference standards, the CBNAAT's sensitivity, specificity, positive predictive value, and negative predictive value are 98.4%, 100%, 100%, and 98.5%, respectively. Among the 63 instances that CBNAAT identified, 7 incidences of rifampicin resistance were additionally identified (11.11%). A strong statistically significant agreement was observed with CBNAAT.

***Index Terms*- TBM, CBNAAT, ZN Smear.**

I. INTRODUCTION

Tuberculosis is the most common infectious disease to cause death globally and ranks among the top 10 causes of death (over HIV/AIDS). In 2019, an estimated 10 million people worldwide were diagnosed with tuberculosis (TB), including roughly 3.2 million women, 1.2 million children, and 5.6 million men. All countries and age groups are affected by TB. Nevertheless, TB can be managed and prevented. Among the two different forms of tuberculosis (Pulmonary and Extra-pulmonary) almost 15% of the index cases reported in 2018 were extra-pulmonary in nature. But the proportion is likely to be higher among the Indian population ranging between l0- 19%. The proportion of such cases might reach up to 50% among the HIV-positive population. [(1)] One of the most difficult TB conditions to treat is tuberculous meningitis (TBM), a severe form of extrapulmonary TB (EPTB) that affects the central nervous system. Although the estimate of the burden of TBM locally or globally is not readily available, different studies conducted over time reported different proportions of cases along with the variability of proportions across locations and overall TB prevalence. In a country like India, the expected and estimated burden must have been towards the higher side. [(2–4)]

Conventionally microscopy of cerebrospinal fluid (CSF) remains the mainstay of diagnosis for TBM for a long time. Gradually different methods of mycobacterial culture evolved with time. Presently new diagnostic technique with Nucleic Acid Amplification (NAA) test offers rapid testing for Mycobacterium tuberculosis along with genotypic detection of rifampicin resistance. [(5–7)] In spite of these different diagnostic modalities, no single test could offer certainty of diagnosis. Variability of sensitivity reported with differences in quality, quantity and processing of CSF sample. [(5–8)] Amid this uncertainty of diagnostic yields studies also reported mortality benefits with improved detection of the disease. [(9)]

Considering the aforesaid facts about TBM and its diagnostic uncertainty, the need for further study has been considered in this part of the world where a dearth of data exists on the matter. In our study, we evaluated the CBNAAT's sensitivity, specificity, positive predictive value, and negative predictive value for detecting tuberculous meningitis at the All India Institute of Medical Sciences, Rishikesh.

II. MATERIAL AND METHODS

A time-bound exploratory study was carried out at All India Institute of Medical Sciences, Rishikesh after taking institutional ethics committee approval (Letter No. AIIMS/IEC/21/196) dated (09/04/2021) and (Letter No. AIIMS/IEC/22/531) dated (20/10/2022) from 1st Jan 2019 to 31st March 2022.

The main objective of this study is to evaluate the role of the Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) in Diagnosing Tuberculous Meningitis in adults. A total of 132 patients aged 18 years or above admitted with suspicion of tuberculous

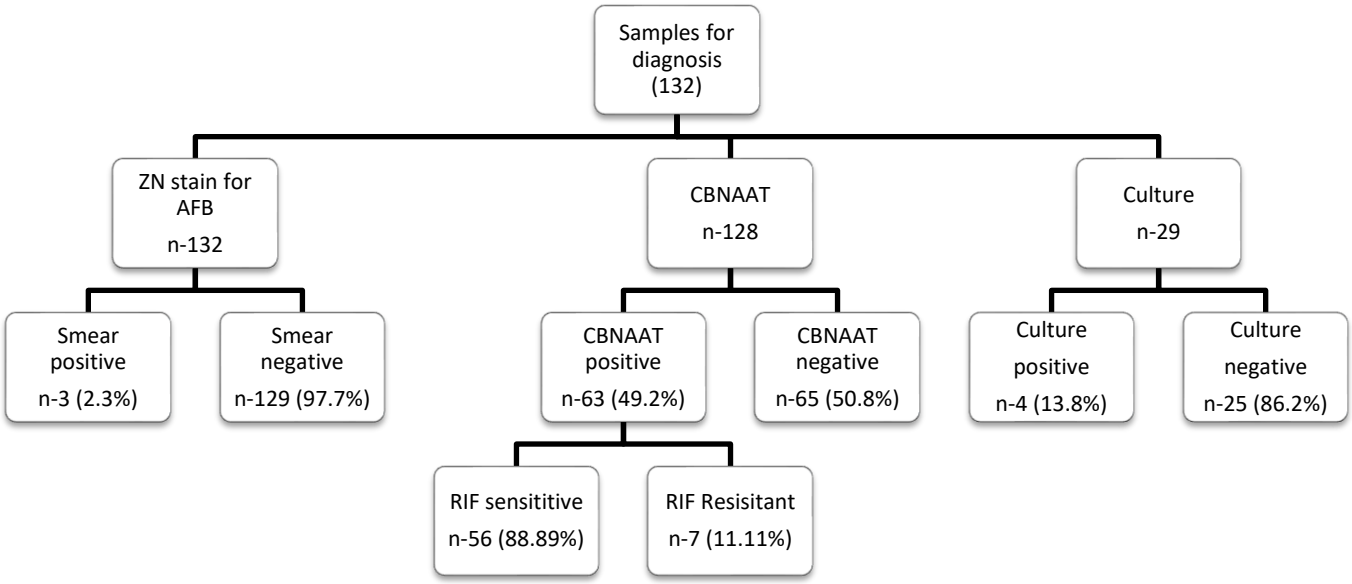
meningitis whose CSF sample was received at the mycobacteriology laboratory were included. Patients with other known causes of meningitis, malignancy, and lymphoma and those unwilling to participate in the study were excluded. Among these 66 were cases and the rest 66 were controls with differential diagnosis as tuberculous meningitis. The samples were collected in a well-labelled leak-proof sterile universal container and were processed within 2 hours without any delay from the time of collection. Smears were examined under a light microscope after ZN staining. Samples were subjected to CBNAAT and culture. Culture was performed only in 29 of the cases out of which 4 were culture positive as owing to the ongoing COVID-19 pandemic, MGIT lab was converted to a COVID-19 extraction lab. In this study setting, due absence of any single test to be identified as a reference standard for the diagnosis of TBM, a composite reference standard with any CSF positivity (with ZN staining, CSF culture and CB-NAAT) will be considered. (10)

Using SPSS software and conventional statistical methods, the collected data was analysed. Calculations were made for the CBNAAT's sensitivity, specificity, positive predictive value, and negative predictive value. Statistics were considered to be significant at $P<0.05$.

III. Results

In accordance with the inclusion and exclusion criteria, 132 cases were collected during the study period, and the samples were then subjected to ZN staining and CBNAAT (Fig. 1).

Fig 1: Methodology workflow



Out of 132 cases included in the study, the maximum number of patients was 18-40 years, i.e., 80(60.6%) followed by 41-60 years, i.e., 35(26.5%). only 17(12.9%) of the cases belonged to the age group >60 Years. Patients' mean age ± SD is 38.55 ± 17.13(Fig 2, Table 1). Predominant samples i.e., 69/132 (52.3%) were comprised of females while 63/132 (47.7%) were male. The male-to-female ratio of the patients was 0.91:1**.** Females were slightly more in number than males (Table 1, Fig 3).

Table 1: Age and gender distribution

Age	**Frequency%**	
18-40 Years	80 (60.6%)	132
41-60 Years	35 (26.5%)	
>60 Years	17 (12.9%)	
Gender		
Male	63 (47.7%)	132
Female	69 (52.3%)	

Fig 2: Age distribution of patients

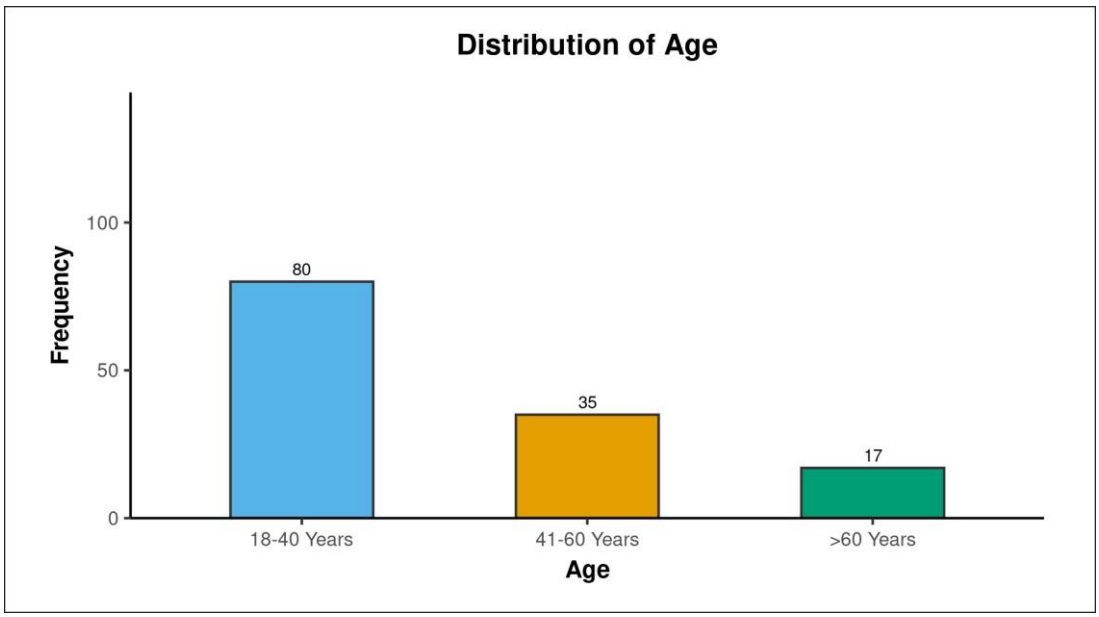
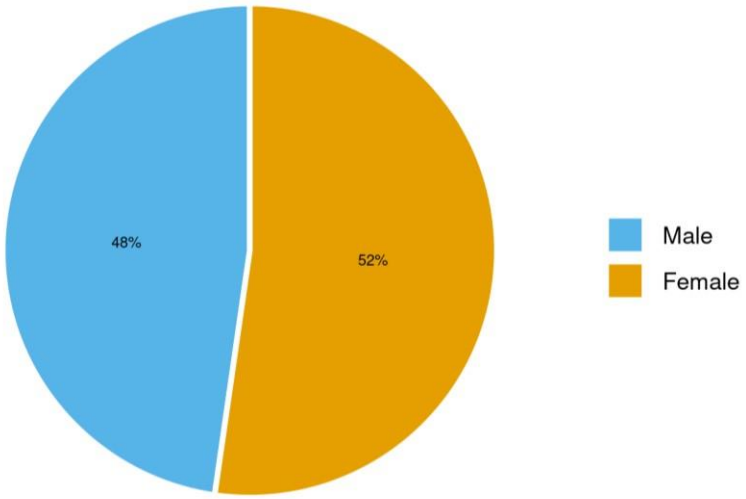


Figure 3: Distribution of Gender in patients



Amongst the 132 cases, only 3 (2.3%) were ZN stain positive. CBNAAT was done in 128 cases of which positivity was observed in 63 (49.2%).

Table 2: Distribution of results of microbiological examinations

Microbiological Examination	Positive	Negative	TOTAL
ZN Stain	3 (2.3%)	129 (97.7%)	132
CBNAAT	63 (49.2%)	65 (50.8%)	128a
AFB Culture	4 (13.8%)	25 (86.2%)	29b

ZN: Ziehl –Neelsen stain, CBNAAT: Cartridge based nucleic acid amplification test, AFB: Acid fast bacilli
a- CBNAAT was performed on 128 cases. It could not be performed on 4 cases due to insufficient amount of sample.
b- Culture was only done in 29 cases due to the COVID pandemic.

The overall sensitivity of CBNAAT was maximum i.e., 98.4% followed by culture (40.0%). Minimum sensitivity was observed for ZN stain (4.5%). The overall specificity for CBNAAT and ZN stain was the same i.e., 100%. Overall, for CBNAAT, strong statistically significant agreement was observed while for culture weak statistically significant agreement was observed. No statistically significant agreement was observed for ZN stain as shown in (Table 3)

Table 3: Diagnostic evaluation of ZN stain and CBNAAT against a composite reference standard

	Diagnostic validity				Cohen's Kappa coefficient (p-value)	*Level of agreement & statistical significance
	Sensitivity	Specificity	PPV	NPV		
ZN Stain	4.5%	100.0%	100.0%	51.2%	0.05(0.080)	No agreement (Statistically insignificant)
CBNAAT	98.4%	100.0%	100.0%	98.5%	0.98(<0.001)	strong agreement (statistically significant)
AFB Culture	40.0%	100.0%	100.0%	76.0%	0.47(0.003)	weak agreement (Statistically significant)

PPV: Positive predictive value, NPV: Negative predictive value, ZN: Ziehl –Neelson stain,
CBNAAT: Cartridge-based nucleic acid amplification test, AFB: Acid-fast bacilli

Among 63 CBNAAT-positive cases, 7 (11.11%) were found to be rifampicin resistant while 56 (88.89%) were rifampicin sensitive.
Table 4: Summary of rifampicin resistance in CBNAAT

	CBNAAT	
Rifampicin resistance	**Frequency**	**Percentage (N=63)**
Positive	7	11.11%
Negative	56	88.89%
Total	63	100%

CBNAAT: Cartridge-based nucleic acid amplification test

IV. Discussion

Tuberculous meningitis (TBM) is a prevalent CNS infection with one of the worst fatality and disability rates of all serious infectious disorders. [11] Early diagnosis and treatment of TBM are essential because it is linked to a high mortality rate and serious neurological aftereffects, particularly in endemic nations like India. The accurate and prompt diagnosis of TBM is complicated by its nonspecific symptoms. [12]

In our study, 60.6% (n=80) of the cases were in the age group of 18-40 years, 26.5% (n=35) were older than 40, and 12.9% (n=17) were older than 60 years old. In trials conducted in India, young adults aged 18-40 years have been reported as the group with the highest incidence rate. [12,13] Similar results were seen in a study done in Turkey by Pehlivanoglu et al[14] where the highest incidence rate was seen in young adults.

This study showed an almost equal number of cases in males and females. With male to female ratio of 0.91:1. This is in contrast with the data published by Kaur et al. [12] showed a higher predominance of cases in males over females. This may be due to the fact that in Uttarakhand female literacy rate is higher in this state as compared to other states of India. [15]

In our study microbiological diagnosis was done with ZN stain, Culture and CBNAAT and a composite reference standard was taken with any CSF positivity. This study showed out of 66 cases, acid-fast bacilli were seen only in 3 (2.3%) cases after ZN staining. The sensitivity of the AFB stain was very poor and was only 4.5%. Both the specificity and positive predictive values of AFB stain were 100%. The negative predictive value was 51.2%. This was in concordance with the study done in northern India by Kusum et al, which showed a sensitivity of 1.81%, specificity and a positive predictive value of 100% while a negative predictive value of 48.7%. [12] This might be due to the paucibacillary character of the TBM as smear positivity requires a bacillary load of 10^4 CFU/ml. [16] The second factor could be the low quantity of the CSF sample received at our laboratory. A prospective international multicentre study by AD Heemskerk AD et. al. showed a higher CSF volume increases the chance of positive results. [17] Similar International studies done in developing countries like South Africa and Indonesia by Bhigjee et al. showed a sensitivity as low as 0% and Chaidir et al showed a sensitivity of 11%. [18,19]

Studies were done in UK and Scotland by Thwaites et al. [8] and Kennedy & Fellon et al. [20] also showed little higher sensitivity of ZN stain of 20% and 37% respectively. Overall, the sensitivity of ZN stain for CSF samples in the diagnosis of tuberculous meningitis remains low (10- 20%) but evidence suggested, a larger volume of CSF sample (>6ml) and slide examination time of 30 minutes can improve the detection rate. [21] Among the three modalities of microbiological diagnosis CBNAAT was found to be the best modality in our study because it has a detection limit of M. tuberculosis bacilli of 131 CFU/ml. [22] In our study CBNAAT was done on 63 (95.45%) cases out of 66 cases. It could not be performed in 3 cases due to insufficient samples.

In this study, among patients with suspected TB meningitis, CBNAAT had a higher positive rate (49.2%) than microscopy (2.3%) and culture (13.8%). This study showed 98.4% sensitivity. Both sensitivity and positive predictive value of the test were 100%. While the negative predictive value was 98.5%. Similar findings were seen in the studies conducted by Chaidir et al. and Kulkarni et al. which showed a sensitivity of 92% and 90% respectively. [19,23] These studies also showed a specificity of 100% similar to our study. However, lower sensitivity (87%) was observed in a study done by Kusum et al. [24] but specificity was 100%.

In our study out of 63 cases that were subjected to CBNAAT, Rifampicin resistance was observed in 7 (11.11%) cases. Fang et al. in their study observed 12 (8.8%) cases of rifampicin resistance MDR-TBM, while 125 cases (91.2%) were non- rifampicin resistance MDR-TBM. No Indian studies were found. This lower number of resistance cases can be due to a small sample size.

V. LIMITATION

Our study is subject to several limitations that warrant consideration. Firstly, the small sample size utilized in our research may limit the generalizability of our findings to a larger population. This constraint can affect the statistical power and precision of our results. Additionally, we encountered a challenge regarding the insufficient quantity of a few cerebrospinal fluids (CSF) samples, which might have limited the depth and comprehensiveness of our analysis for those specific cases. Furthermore, the COVID-19 pandemic resulted in a reduced number of available samples, further restricting the overall sample size and potentially introducing selection bias. Therefore, while our study provides valuable insights, these limitations should be considered when interpreting and generalizing the results to a broader context.

VI. ACKNOWLEDGEMENT

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