



## Original Research Article

## Comparative evaluation of Conventional and Molecular methods in diagnosis of Tuberculous Meningoencephalitis

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## ABSTRACT

**Background:** Over 1.2 million cases of bacterial meningitis are estimated to occur worldwide each year. World Health Organization (WHO) estimated 9 million people developed tuberculosis in 2013, and 1.5 million died. Tuberculous meningitis (TBM) is still one of the common infections of central nervous system (CNS) and poses significant diagnostic and management challenges, more so in the developing world. The main reason for the spread of tuberculosis is poverty, with resulting homelessness, overcrowding, malnutrition, HIV, excessive alcohol use, diabetes and breakdown of public health infrastructure.

**Materials and Methods:** CSF samples were collected aseptically and processed with an aim to identify and isolate Mycobacterium tuberculosis from clinically suspected cases of chronic meningoencephalitis and compare their conventional and molecular methods of diagnosis.

**Results:** The study group included 197 patients clinically diagnosed as meningoencephalitis. Out of which, 117 had features of chronic meningoencephalitis and were subjected to Z-N staining, culture on LJ media and CBNAAT testing. From 117 cases, 21 cases were AFB positive, 20 cases culture positive and 36 cases were positive for Mycobacterium tuberculosis.

**Conclusion:** CBNAAT (molecular testing) is a better diagnostic tool for diagnosing tuberculous meningitis.

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### 1. Introduction

The term Meningoencephalitis involves inflammation of meninges, the subarachnoid space and the brain parenchyma.<sup>1</sup> As meninges, subarachnoid space, and the brain parenchyma are all together involved in the inflammatory reaction and often difficult to reliably differentiate meningitis and encephalitis clinically hence 'meningoencephalitis' is the most appropriate term.<sup>2</sup>

Determining the etiology of meningoencephalitis is difficult. The most common bacteria that cause meningitis presently are *H. influenzae*, *N. meningitidis*, *S. pneumoniae* and *Listeria monocytogenes*. Chronic meningitis and meningoencephalitis are mostly caused by *Mycobacterium tuberculosis*, *Cryptococcus neoformans* and parasites like

*Acanthamoeba*, *Naegleria* etc.<sup>3,4</sup>

Tuberculosis meningitis (TM) represents nearly 2% of all the tuberculosis cases worldwide.<sup>5</sup> This form of presentation is particularly important because of the significant high rate of mortality and disability.<sup>6</sup> A delay in diagnosis usually leads to high morbidity or death.<sup>3</sup>

Tuberculous meningoencephalitis may present as subacute meningitis or as chronic meningitis. The clinical manifestations in subacute meningitis typically have an unremitting head-ache, low grade fever and lethargy for days to several week, Whereas in cases of chronic TBM the clinical manifestations are mainly due to obstruction of CSF pathways, and presents with headache or back pain, symptoms of raised intracranial pressure, including headache, vomiting, apathy or drowsiness, gait instability, papilledema, visual loss, impaired up gaze, or palsy of sixth cranial nerve.

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## 2. Materials and Methods

The present study was conducted in the department of Microbiology, M.K.C.G Medical College, Berhampur in collaboration with department of Paediatrics and Medicine.

### 2.1. Type of study

Prospective study.

### 2.2. Period of study

The study was carried out over a period of 24 months, from February 2018 to January 2020.

### 2.3. Study group

Clinically diagnosed cases of meningoencephalitis admitted in the department of Paediatrics & Medicine, M.K.C.G Medical College and Hospital were included in the study. This study was approved by IEC of M.K.C.G Medical College and Hospital.

### 2.4. Selection of cases

#### 2.4.1. Inclusion criteria

Clinically diagnosed meningoencephalitis patients who had following clinical features like Fever, Headache, Vomiting, Neck stiffness, altered or reduced level of consciousness, convulsions, seizure, facial weakness, double vision, visual loss, Photophobia, papilledema, Poor sucking and irritability were included in the study.

#### 2.4.2. Exclusion criteria

Clinically diagnosed cases of

1. Cerebral malaria.
2. Metabolic encephalopathy.
3. Altered sensorium due to traumatic or narcotic abuse.
4. Other conditions of fever.

Though these clinical entities have features of meningoencephalitis, they were excluded from the study group.

**Samples:** Cerebrospinal fluid (CSF) and other samples were collected from all patients. However, only CSF samples were included under the scope of this study for conventional and molecular methods of testing.

### 2.5. Specimen collection and transport

#### 2.5.1. Cerebrospinal fluid

Under strict aseptic precautions, lumbar puncture was done on clinically diagnosed cases of meningoencephalitis, preferably before initiation of antimicrobial therapy. 3 to 5 ml of CSF for adults and 1 to 2 ml for children were collected in sterile screw capped containers. Reused or

unsterile containers were not used as they may contain dead bacteria from previous samples, which may lead to erroneous findings.

The specimens were transported to the Microbiology laboratory without any delay. If delay was unavoidable, CSF was kept in an incubator (37 °C).

#### 2.5.2. Sample processing

**Cerebrospinal fluid:** The samples were processed immediately. It was examined with naked eye for the presence of turbidity or any signs of contamination with blood from the puncture wound. The sample was divided into two parts.

The first part was subjected to centrifugation at the speed of 1500 X g for 15 minutes. The centrifuged deposits, was mixed thoroughly and was then used for routine staining including Z-N stain and inoculation onto L-J slants.

The 2<sup>nd</sup> part of the CSF samples were centrifuged at 4,000 X g for 15 min. Supernatant was removed to leave deposit which was subjected for molecular study (gene expert systems)

1. **Z-N staining** of CSF deposit in symptomatic cases was done to detect the presence of acid fast bacilli in samples. Ziehl-Neelsen smears were prepared using standard methods with two modifications. First, the smear was layered, with two drops of CSF deposit applied. The layered smear was then stained according to standard procedures. Second, the ZN smear was meticulously examined for up to 30 min under a 1,000X magnification before being recorded as negative. Observation of a single acid-fast bacillus was considered a positive result.
2. **Bacterial Culture:** The centrifuged CSF deposit was mixed thoroughly and inoculated on LJ media and were observed once in a week for the first month and then twice a week in the next month. The LJ slants were observed for 8 weeks before declaring negative.
3. **Molecular test :Cartridge based nucleic acid amplification test (Gene Xpert MTB/RIF) :** The Gene Xpert MTB/RIF test (Cepheid) is a closed-cartridge based system that is easy to operate and gives results in approximately 2 h time period. The test is based on a real-time hemi-nested PCR test which detects the presence of M. tuberculosis complex bacilli.

Upon receipt in the TB laboratory, all CSF samples were centrifuged at 4,000 X g for 15 min. Supernatant was removed to leave deposit. A 200 µl portion of the deposit was re-suspended in phosphate-buffered saline to a 500 µl volume. The sample reagent supplied with the test (1.5 ml) was then added. The mixture was vortex for 30 sec to ensure all bacteria were re-suspended. The sample was left to stand for 15 min, as per the manufacturer's instructions, with intermittent manual shaking. The solution was then

transferred to the Xpert cartridge using a Pasteur pipette, and the cartridge was loaded onto the Xpert machine for analysis.

### 3. Results

A total number of 197 patients were included in the study group constituting 126 children and 71 adults. Out of 197 cases, 125(63.5%) were males and rest 72(36.5%) were females. The study group was further categorized into acute and chronic meningoencephalitis based on whether duration of signs and symptoms was > 4 weeks. In this study, clinically diagnosed cases of chronic meningoencephalitis were 117(59.4%) and acute meningoencephalitis cases were 80(40.6%) of total cases (Table 1).

In ZN staining 21 (17.9%) smears were AFB positive and Mycobacterium tuberculosis was isolated in culture in 20 (17.1%) cases (Figure 1). In CBNAAT, Mycobacterium tuberculosis was detected in 36 cases (30.7%) (Figure 2)

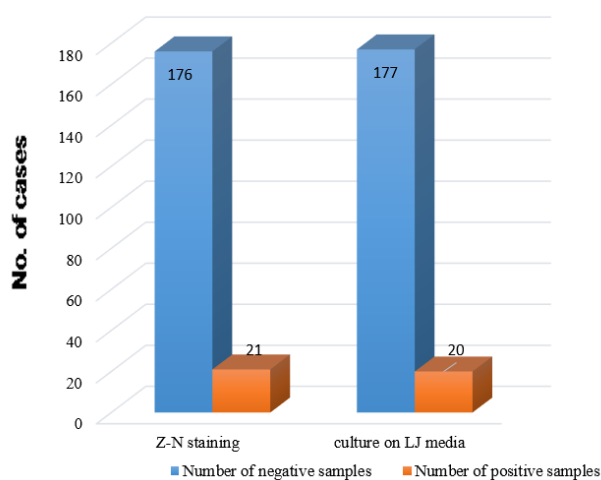


Fig. 1: Detection of Z-N staining and culture on LJ media (n=117)

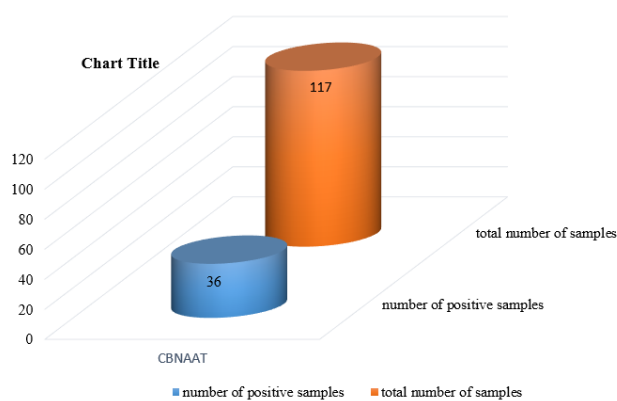


Fig. 2: Detection of Mycobacterium tuberculosis by CBNAAT in CSF deposit (n=117)

### 4. Discussion

This prospective study included 197 patients who were clinically diagnosed as meningoencephalitis. In the study group, 126 cases belong to the paediatric and 71 cases belong to the adult age group. In both the age groups males accounted for more than females. The reason for male preponderance is not known although increased environmental exposure, hormonal influences, and genetic predisposition have been postulated to be contributing factors.

In our study out of 197 cases, 117 patients had presented with signs and symptoms of chronic meningitis with brain parenchyma involvement whereas rest 80 had presented as acute meningoencephalitis. Among the 117 patients of chronic meningoencephalitis, 70 patients (59.8%) were children and 47 (40.2%) were adults. Lumbar puncture and examination of CSF by routine staining including Z-N stain, along with culture on LJ media. On Z-N staining, acid fast bacilli were seen in 21 (17.9%) CSF samples which were further subjected to identification of bacteria by conventional culture and CBNAAT methods. The findings of our study matches to the findings of a study by Hopewell et al (2005),<sup>7</sup> in which acid fast bacilli were seen in CSF smears in about 10% to 20% of cases in those with TBM, although this figure varies considerably. The values in recent reviews were 12.5% in a review by Verdon R et al. (1996).<sup>8</sup>

A total of 20 samples from the 197 cases had yielded Mycobacterium growth on L-J media. Culture positive in CSF samples is still considered to be a gold standard as this method provides a further lead in accessing the drug susceptibility of the grown mycobacterial cultures. Mycobacterium tuberculosis species was isolated from all the 20 isolates. Improper technique of lumbar puncture, prior antibiotic therapy, delay in sample transport and culture, low bacterial load, disease by some fastidious and anaerobic organisms and presence of autolytic enzymes in CSF may be the possible reasons for low isolation.<sup>9</sup>

All the 117 cases clinically diagnosed to chronic meningoencephalitis were subjected to CBNAAT test out of which 36 (30.7%) cases were positive for Mycobacterium tuberculosis. CBNAAT test has higher sensitivity for detection of Mycobacterium tuberculosis i.e. 30.7% as compared to Z-N staining 17.9% and culture 17%. One Vietnamese study compared 104 patients treated for TBM on clinical grounds and the results of initial CSF microscopy, culture, and PCR. They report the sensitivities of PCR to be 32%, culture 17% and microscopy 1%.<sup>10</sup> Similar to our study both the studies has higher detection rates of Mycobacterium tuberculosis by PCR technique, but the detection rate varies widely.

**Table 1:** Acute Vs Chronic meningoencephalitis in the study group (n=197)

Age group	Acute Meningoencephalitis	Chronic Meningoencephalitis	Total
Children (n=126)	56	70	126
Adult (n=71)	24	47	71
Total	80 (40.6%)	117 (59.4%)	197(100%)

## 5. Conclusion

From the above study, we conclude CBNAAT has a better prospect for detection of Mycobacterium tuberculosis in TBM cases. Meningoencephalitis is a medical emergency that requires prompt assessment and treatment. They have a considerable mortality, morbidity and serious long term sequelae despite of advances in medical care. Hence early and accurate diagnosis with the administration of appropriate antibiotics remains the key element of management.

## 6. Source of Funding

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## 7. Conflict of Interest

The authors declare they have no conflict of interest.

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