Comparison of Gene XPERT MTB/RIF Assay and Conventional diagnostic modalities for the diagnosis of tubercular meningitis in Pediatric Patients

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Abstract

Introduction:- *Tuberculosis(TB) remains one of the deadliest communicable diseases. India is the highest TB burden country in the world, accounting for about 26% of the global prevalence and estimated incidence being 1.8 million cases. While pulmonary tuberculosis is the most common presentation, extra-pulmonary tuberculosis (EPTB) is also an important clinical problem. Among various forms of extrapulmonary TB, tubercular meningitis (TBM) is the most severe form of EPTB. Globally, almost half a million children become sick with tuberculosis every year, 20-30%being affected by EPTB. TBM constitutes a small portion of the total reported TB cases (around 1-10%) Diagnosis of TBM requires demonstration of tubercle bacilli (Acid fast bacilli) in CSF taken by Lumbar puncture. Conventional methods that includes microscopy and culture are used for demonstration of tubercle bacilli but these methods are less sensitive. More recently, the WHO endorsed the Gene Xpert for rapid identification of disease. The aim of the study was to compare Gene Xpert MTB/RIF Assay and various conventional modalities in the diagnosis of tubercular meningitis in pediatric patients*

Material and methods

The present study was carried out in Microbiology Department in collaboration with Chest & TB hospital and pediatric wards of Guru Nanak Dev Hospital, Amritsar. The study was conducted from January 2020 to January 2022. Total 116 children (Upto 14 years of age) clinically suspected of tubercular meningitis and attending indoor service at our institute were included in our study.

Results

A total of 116 children were enrolled. Maximum number of patients were below 1 year of age. In this study, males outnumbered the females. All the CSF samples were examined by conventional methods and Gene Xpert. Out of 116 CSF sample, 3 were positive by gene Xpert and one CSF sample was positive by fluorescent microscopy and culture by LJ media.

Keywords: *Cerebrospinal fluid, EPTB, Tubercular meningitis*

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I.   Introduction

Tuberculosis (TB) is a serious health problem and is one of the top 10 causes of death. India is the highest TB burden country in the world accounting for about 26% of the global prevalence and estimated incidence being 1.8 million cases[1] [2]. TB is caused by the bacillus Mycobacterium tuberculosis, which was discovered by Robert Koch in 1882[3]. Tuberculosis is primarily an airborne infection of lungs causing pulmonary disease. The dissemination of TB bacilli accounts for the extrapulmonary TB resulting in TB lymphadenitis, skeletal TB, miliary TB, genitourinary TB, gastrointestinal TB, Tubercular meningitis (TBM).

Globally, almost half a million children became sick with tuberculosis every year, 20-30% being affected by extrapulmonary TB (EPTB). Almost 10-20% of the children with TB live in high TB burden countries. Tubercular meningitis (TBM) most lethal form of EPTB constitutes a small portion of the total reported TB cases (around 1-10%)[4], it causes a disproportionate amount of suffering with higher rates of mortality and morbidity especially in young children.

The clinical onset of TBM may be acute, sub-acute or gradual and is characterized by non-specific symptoms in early stages, such as headache, low grade fever, symptoms related to pulmonary TB[5]. Children with more advanced disease may have signs of meningeal irritation, raised intracranial pressure, cranial nerve palsies, neurological deficits, altered sensorium[6].

The diagnosis of EPTB is challenging because of its paucibacillary nature, lack of specific signs symptoms and often negative acid fast bacilli smear of biological specimen[7]. The various methods used in diagnosis of TBM include CSF cellular and biochemical analysis, microbiological confirmation in CSF and other supportive testing such as molecular methods and neuroimaging. CSF findings in TBM consist of leukocytosis with lymphocyte predominance, protein elevation and decrease in CSF glucose[6].

II.   Aims & Objectives
To compare Gene Xpert MTB/RIF assay and conventional diagnostic methods i.e. staining methods and culture on LJ medium in clinically suspected pediatric cases of Tubercular meningitis.

III.   Material And Methods
A prospective observational study was carried out in Microbiology Department, Government Medical College, Amritsar in collaboration with Pediatric department of Guru Nanak Dev Hospital and Chest & TB hospital, Amritsar. A total of 116 cases of clinically suspected of tubercular meningitis admitted in pediatric wards of our institute were included in the study.

CSF sample (2-3 ml) was collected aseptically from these cases by performing lumbar puncture under aseptic conditions in sterile leak proof containers. A part of it was sent to biochemistry department for biochemical analysis and pathology department for cytological findings. Rest of the CSF sample was transported to microbiology department. CSF sample was divided into 2 parts. The first portion was centrifuged and part of sediment was used. After macroscopic examination, the CSF was subjected to ZN staining, Fluorescent staining, part of sediment was inoculated onto LJ media and the uncentrifuged second part was sent for Gene Xpert MTB/RIF assay.

IV.   Results
A total of 116 CSF samples of patients (upto 14 years of age) clinically suspected of Tubercular Meningitis were collected from various pediatric wards of Guru Nanak Dev Hospital, Amritsar and were processed.

Table 1:Age and sex distribution of cases in the patients suspected of Tubercular meningitis (n=116)

Age group(Years)	Sex		Total(%)
	Male No(%)	**Female No(%)**	
<1	33(28.4%)	19(16.4%)	52(44.8%)
1-5	20(17.3%)	18(15.5%)	38(32.8%)
6-10	14(12%)	5(4.2%)	19(16.4%)
11-14	2(1.7%)	5(4.3%)	7(6%)
Total	69(59.4%)	47(40.6%)	116(100%)

The present study constituted the patients upto 14 years of age group with mean age found to be 2.78 years. Maximum number of the cases (n=52) were in the age group <1year followed by 1-5years

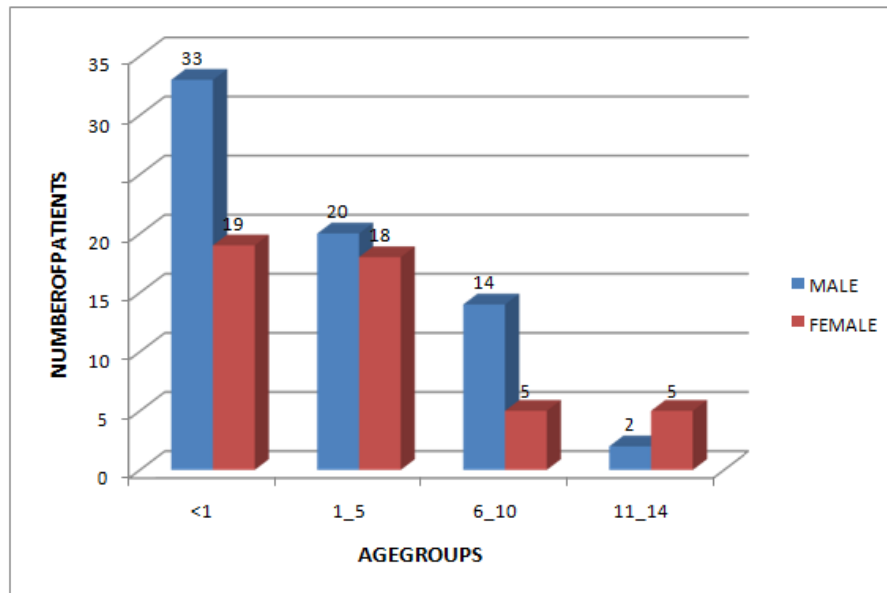**Figure 1: Age and sex distribution of cases in patients suspected of Tubercular meningitis**

Table 2: History of positive Contact among patients suspected of Tubercular meningitis (n=116)

History of positive contact	Number	Percentage (%)
Positive	13	11.2%
Negative	103	88.8%

In this study, it was found that history of positive contact was present in11.2% (n=13) of suspected cases, while the rest had negative history (88.8%).Positive contacts were mainly relatives or neighbours.

Clinical presentation

Table 3: Clinical presentation of the patients suspected of Tubercular meningitis

Presentation	Number	Percentage (%)
FEVER	102	87.9%
HEADACHE	30	25.9%
VOMITING	34	29.3%
ALTERED SENSORIUM	26	22.4%
SEIZURES	49	42.2%
LOSSOF APPETITE	34	29.3%
IRRITABILITY	35	30.2%
NECKRIGIDITY	5	4.3%

In the present study, the most common presentation by the patients in decreasing frequency were Fever (n=102; 87.9%), Seizures (n=49; 42.2%), vomiting (n=34; 29.3%), irritability (n=35; 30.1%), anorexia (n=34; 29.3%), headache (n=30; 25.9%), altered sensorium (n=26; 22.4%) and neck rigidity (n=4; 3.4%).

Figure 2: Distribution of different clinical features among patients suspected of Tubercular meningitis
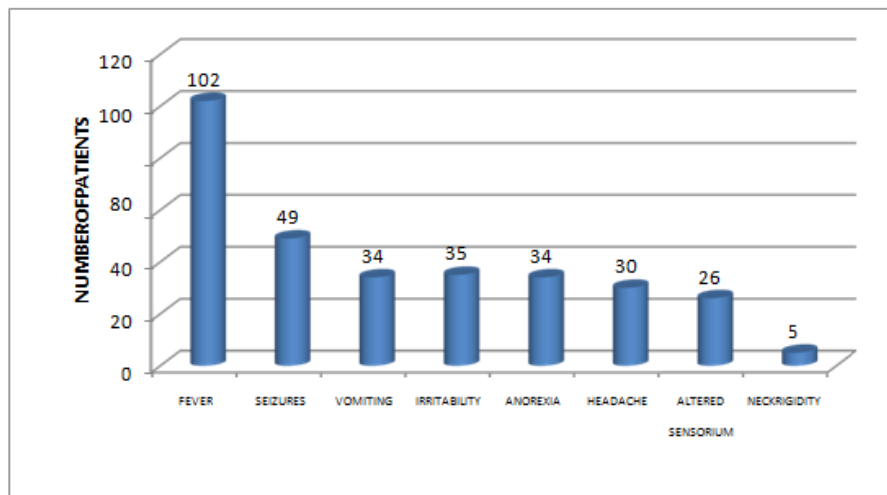
Investigations

Table 4: Hematological parameters associated in patients suspected of Tubercular meningitis (n=116)

Hematological Investigations	Number	Percentage (%)
Hemoglobin g/dl		
<10g/dl	52	44.8%
10or>10g/dl	64	55.2%
TLC cells/cu mm		
<9000cells/cu mm	33	28.4%
>9000cells/cu mm	83	71.6%
Neutrophilia (55-70%)	20	17.2%
Lymphocytosis (20-40%)	13	11.2%

In this study, Leukocytosis i.e. raised Total leukocyte count (TLC) (71.6%) was most predominant altered parameter followed by Anemia i.e. decrease in hemoglobin (44.8%), Neutrophilia i.e. increased neutrophils (n=20; 17.2%) and Lymphocytosis i.e. decreased lymphocytes (n=13; 11.2%)

Biochemical findings of CSF:

Table 5: CSF Protein analysis in patients suspected of Tubercular meningitis (n=116)

CSF PROTEIN LEVEL	NO. OF PATIENTS
Normal	83(71.6%)
Increased	33(28.4%)

In most of the suspected cases of TBM, CSF protein level was found to be normal (71.6%) while 28.4% showed increased CSF protein levels

Figure 3: CSF Protein analysis in patients suspected of Tubercular meningitis (n=116)
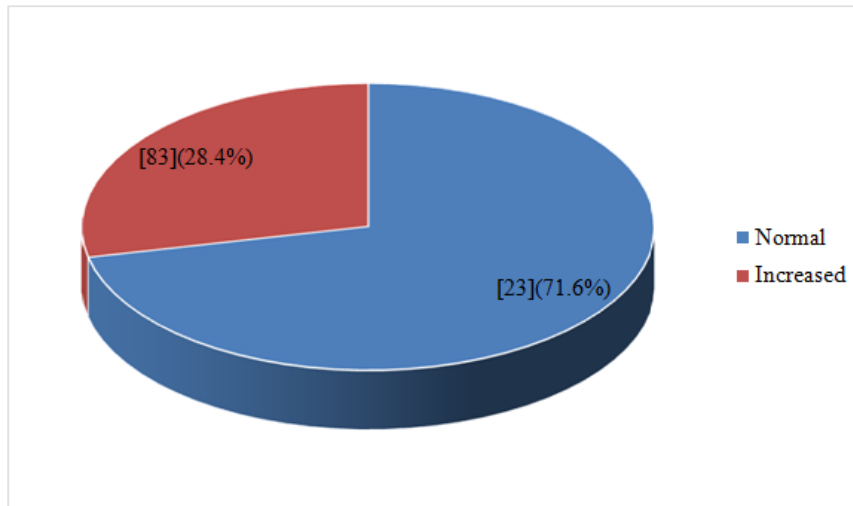

TABLE 6: CSF Glucose analysis in patients suspected of Tubercular meningitis (n=116)

Sr. No.	CSF Glucose level	No. of patients (n=116)
1.	Normal	93(80.2%)
2.	Decreased	20(17.2%)
3.	Increased	3(2.6%)

Majority of suspected cases of TBM (80.2%) was having normal CSF Glucose levels however decreased and increased CSF glucose level was found in 17.2% &2.6% of cases respectively.

Figure 4: Bar diagram showing CSF Glucose analysis in patients suspected of Tubercular meningitis (n=116)
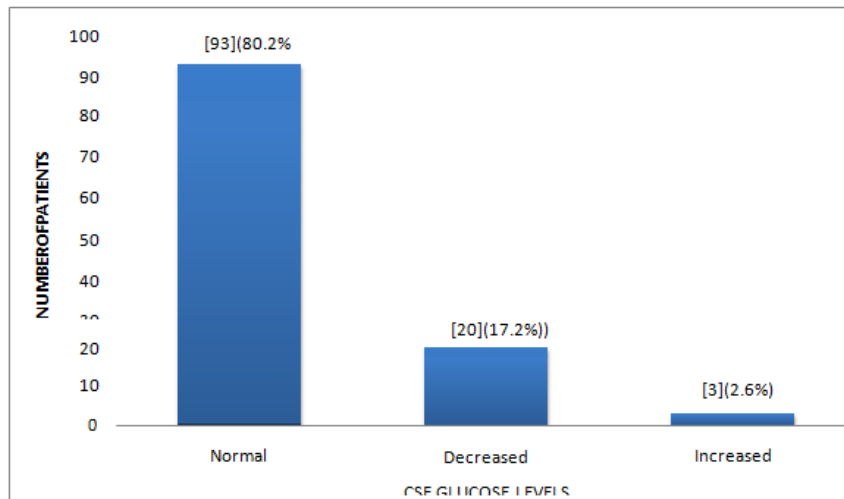

TABLE 7: Distribution of MRI brain findings in patients suspected of Tubercular meningitis (n=116)

Sr. No.	MRI Findings	No. of patients (n=116)
1.	Basal meningeal enhancement	6(5.2%)
2.	Hydrocephalus	5(4.3%)
3.	Tuberculoma	8(6.9%)
4.	Normal brain study	100(86.2%)

In the present study, 6.9% of the patients had Tuberculoma followed by basal meningeal enhancement (5.2%) and hydrocephalus (4.3%).

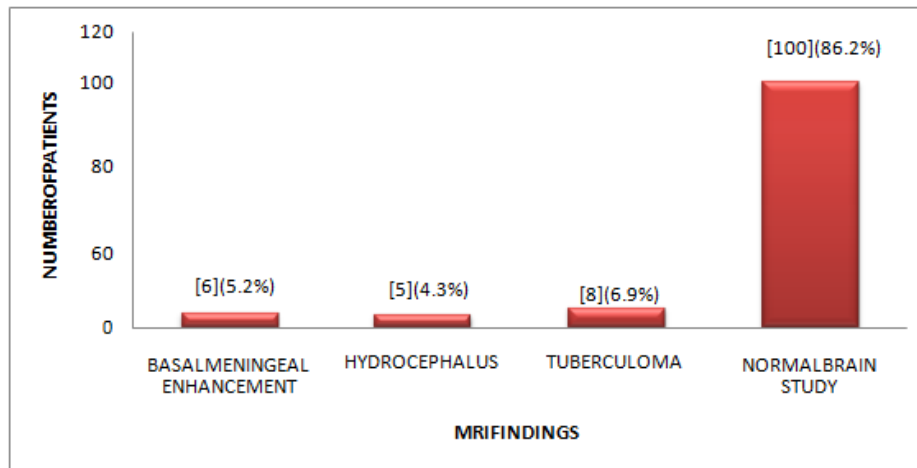**Figure 5: Diagram Showing Distribution of MRI Brain Findings in a Study Population (n=116)**

Table 8: Results of various methods used in study

Interpretation	CBNAAT		Fluorescent Microscopy		LJ culture		ZN staining	
	Number	Percentage (%)	Number	Percentage (%)	Number	Percentage (%)	Number	Percentage (%)
Negative	113	97.4%	115	99.2	115	99.2	116	100
Positive	3	2.6%	1	0.8	1	0.8	0	0
Total	116	100	116	100	116	100	116	100

This study compared conventional culture on LJ medium, ZN staining, Fluorescent microscopy and Gene Xpert. Out of 116 patients enrolled in the present study, 1(0.8%) were positive by fluorescent microscopy, 1(0.8%) by LJ culture 3(2.6%) by Gene Xpert.

V.   Discussion

Tubercular Meningitis (TBM) is undoubtedly the most serious and devastating form Of EPTB. The case fatality rate for untreated TBM is almost 100% and those who survive after treatment are left with severe neurological sequelae[8]. Diagnosing TBM accurately is still challenging due to paucibacillary nature of CSF.

The present study included 116 total children upto 14 years of age. . In this study, Gene Xpert positive patients were 3 in number which were <1 year of age. In a study conducted by Bang N D et al in the year 2016[9] and another study conducted by Daniel B D et al in the year 2019[6] reported the peak incidence of TBM in <5 years of children which is in correlation with our study. Different results were depicted by an another study conducted by Rai A et al in the year 2021[10],in which the peak incidence of TBM was in the age group 5-10 years. It could be due to more number of children (50.9%) between the age group of 5-10 years.

In this study, males outnumbered the females in the total samples. 58.6% of total samples were received from males while 41.4% of total samples were received from females. Similar results were depicted in a study by Malik V S et al[11] and Thilothammal N et al[12] which is in accordance with our study. In another similar study by Dahiphale R et al[13] showed female predominance. Among 3 positive cases by Gene Xpert, 2 were male and 1 was female.

In the present study, the most common complaints by the patients in decreasing frequency were fever (n=102; 87.9%), seizures (n=49; 42.2%), followed by irritability (n=35; 30%) vomiting, loss of appetite (n=34;

29.3%), headache (n=30; 25.9%), altered sensorium (n=26; 22.4%), and neck rigidity (n=4; 3.4%). In a study by Kaur H et al[14], Dahiphale R et al[13] and Thilothammal N et al[12], they reported similar symptoms with maximum frequency of fever followed by seizures, vomiting and anorexia. In contrast to another study conducted by *Lavanya S R et al*[15] mentioned findings like fever in 100%, altered sensorium in 58%, headache in 41%, vomiting in 46%. This study involved patients >18 years of age.

In this study, it was found that positive history of contact was present in11.2% (n=13), while the rest had negative history (88.8%) . Similar results were depicted in the study by Kaur H et al[14]. In contrast to another study by S.R. et al[15] that showed history of contact in 24% of patients.

In this study, 44.8% of the patients had anemia, leukocytosis in 71.6% and lymphocytosis in 11.2%. Anemia is highly prevalent in tubercular patients and also considered as important risk factors as it predisposes the individual to certain infections like TB. Leucocytosis and lymphocytosis are frequent reported abnormalities among TB patients[16].

All of the positive cases, 3(100%) cases had raised protein in CSF and decreased glucose levels. A study conducted by S.R. et al[15] reported normal protein level in 36 patients and increased protein level in 64 patients out of 100 patients. Also same study reported normal glucose level in 81%, increased glucose level in 5% and decreased glucose level in 14% patients. Decreased CSF glucose levels commonly encountered biochemical derangements during the acute stage of childhood tubercular meningitis.

In the present study, 8(6.9%) cases had tuberculoma, 6(5.2%) cases had meningeal enhancement, 5 (4.3%) cases had hydrocephalus and rest had normal study. Out of 3 cases positive by GeneXpert, 2 had abnormal neuroimaging findings. A study conducted by Chatterjee et al mentioned tuberculomas and hydrocephalus as most common presentation in tubercular meningitis cases[17].

ZN staining is rapid and economical but it detects AFB only when the load is above 10^5 bacteria/ml[18]. Hence it is less sensitive and less specific in case of CSF samples due to its paucibacillary nature. In the present study, none of the CSF sample demonstrated AFB on ZN smear microscopy[19]. It could be due to less volume of samples obtained. Also repeated large volume samples are required for ZN microscopy of CSF samples to came out to be positive. A study conducted by Bala and Goyal et al also reported 0% ZN smear microscopy positivity in 110 CSF samples[20]. Another study by Iqbal et al reported only 2 samples positive out of 450 when subjected to ZN staining i.e sensitivity of 2.86%[21].

Recently the fluorescent microscope using the auramine-O staining has been found to have an increased sensitivity by 10%[22]. In this study, 1 out of 3 Gene Xpert positive cases was positive by fluorescent microscopy. It has the advantage of being less laborious, since bacteria fluoresce in front of a dark background and are easier to count[23]. In a study by Adarsh et al reported 4% positivity by fluorescent microscopy more than ZN staining positivity of 1.3%[22].

The "gold standard technique" Culture cannot detect MTB in CSF as it require atleast 100 bacilli/ml of sample. Hence considered less sensitive. In this study, only 1 sample came out to be positive by culture. It could be due to less volume (1-2 ml) of samples obtained. Also repeated large volume samples (6-10ml) are required for culture of CSF samples for better results. In a study conducted by Kandhakumari et al reported all 43 CSF samples negative by culture[18]. In contrary Venkataswamy et al reported a little higher limit of 4% samples positive by LJ culture[24].

Gene Xpert is considered an important breakthrough in the fight against TB. It has potentially come up as a potentially important method for the rapid diagnosis of TBM[8]. It is single use closed-cartridge based system that does sample decontamination, automated nucleic acid amplification and fluorescence based quantitative PCR[25]. It accurately detect both TB and resistance to rifampicin in less than2 hours[26].

In this study, 3 (2.6%) patients were positive by Gene Xpert and 113 (97.41%) were negative. In a study conducted by Bala and Goyal et al[20], 5 patients (4.55%) were positive by Gene Xpert. Another study by Rai et al[10] reported 9 (16.4%) cases positive by Gene Xpert. Gene Xpert results for extrapulmonary samples especially CSF samples have shown variable results. It could be due to presence of scanty bacteria in CSF sample. Most of the clinical and neurological manifestations or complications in TBM are due to inflammatory immune response rather than direct damage by MTB itself[27]. Also negative CBNAAT does not rule out TB and final decision to start ATT (Anti-tubercular drugs) should be based on clinical, biochemical and radiological profile especially in TBM[20].

Diagnosis of EPTB is challenging. Moreover, conventional methods have long turnaround time and less sensitivity in case of EPTB. So, Gene Xpert has been shown to be rapid, with a result for TB and RIF resistance in under 2 h is not prone to cross-contamination; requires minimal biosafety facilities can be performed by technicians with little training; and has a high sensitivity in smear-negative pulmonary TB and EPTB[28]. These characteristics also make it a potentially attractive tool for extrapulmonary specimens.

With Gene Xpert considered as reference standard, the sensitivity, specificity, PPV and NPV of ZN staining was 0.00%, 100%, 0.00%, 97.41% respectively. The sensitivity, specificity, PPV and NPV of Fluorescent staining was 33.33%, 100%, 100%, 98.26% respectively. The sensitivity, specificity, PPV and NPV of LJ culture was 33.33%, 100%, 100%, 98.26% respectively.

A study conducted by Iqbal et al depicted 2.86% sensitivity, 100% specificity, 100% PPV and 84.89% NPV of ZN smear microscopy. The sensitivity, specificity, PPV and NPV of LJ culture 49%, 100%, 100% and 89.9% respectively. The sensitivity, specificity, PPV and NPV of Gene Xpert 63%, 100%, 100% and 93.6% respectively[21].

The present study demonstrated Gene Xpert has good potential for the diagnosis of TBM where bacterial load in sample is scanty. With detection of MTB and rifampicin susceptibility results in 2 hours, it can definitely help in early diagnosis and early treatment initiation in TBM patients. Conventional techniques can be used simultaneously for microbiological confirmation.

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