Original Research Article

Molecular Diagnosis of Mycobacterium Tuberculosis in CSF Sample of Suspected TB Meningitis Patients and their Drug Resistance Pattern by Geno type MTBDR plus Line Probe Assay Method at Tertiary Care Centre of Western Rajasthan

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Conflict of interest: Nil

Abstract

Introduction: Tuberculous meningitis (TBM) is one of the most serious clinical manifestations of tuberculosis with distressing levels of neurological morbidity.

Aim: To determine the positivity of tuberculosis in CSF sample of suspected TB meningitis patients by Mycobacterium Growth indicator tube (BACTEC-MGIT960) liquid culture and first and second line drug resistance pattern by LINE PROBE ASSAY method.

Methods: A cross-sectional study carried out from 110 CSF samples aged above 18 years received in TB & DST Lab, Department of Microbiology, IDI block, Kamla Nehru Chest, Hospital, Dr S.N. Medical college Jodhpur, Rajasthan during August 2022 to January 2023.

Result: Age group 41-50 yrs old patients were highest in number followed by >61 yrs old. Only 4.5% are ZN staining positive, MGIT culture were 23.64% and MTP64 card test was positive in 9.1% samples. ZN stain and MGIT results in relation to MTP64 results were found to be statistically significant (p<0.0001). All 10 samples processed for MTBDRplus genotype LPA, all are sensitive to first line ATT.

Conclusion: This study reveals that all the samples which are MTP 64 card positive are sensitive to first line ATT.

Key words: Tuberculous meningitis, BACTEC-MGIT960, LINE PROBE ASSAY.

Introduction

Among patients with tuberculosis, approximately 1 to 5 percent are complicated by CNS TB. HIV is an important risk factor for CNS TB. Tuberculous meningitis (TBM) is one of the most serious clinical manifestations of tuberculosis with distressing levels of neurological morbidity. The disease is associated with very high mortality (30%) in cases of infection with fully sensitive organism and much higher mortality and morbidity in infection with drug-resistant organisms.[1] The burden of drug resistance in TBM patients is largely unknown in India, drug resistance is increasing due to improper diagnosis inappropriate treatment and inadequate monitoring and control.[2] India and China account for nearly 50% of the global burden of multi drug resistant (MDR) cases, India is having the highest number.3 Early and

rapid diagnosis of drug resistance in useful clinical time frame is of most important Conventional culture and drug susceptibility testing on solid culture systems is a time consuming process and suffers from higher contamination rates. Clinical features of TBM includes Headache, fever, altered sensorium triad of meningitis. Features that may help distinguish tuberculous meningitis from bacterial meningitis includes Subacute presentation, Presence of neurologic symptoms and Presence of cranial nerve palsies. The line probe assay is a strip-based technology that has sufficient sensitivity and specificity to promptly identify RIF and INH drug resistance in MTB isolates. The BACTEC MGIT 960 system (Becton Dickinson) are fully automated, continuously monitoring, walk-away systems. The BACTEC MGIT 960 system is a non-invasive, nonradiometric system that uses the same technology used by manual MGIT and the BACTEC 9000 MB system. For early and rapid diagnosis of drug resistance in useful clinical time frame is of most important not only for management of TBM but also for reducing spread of MDR tuberculosis, Also such studies are rare from India, specially in Rajasthan.

Aim: To determine the positivity of tuberculosis in CSF sample of suspected TB meningitis patients by Mycobacterium Growth indicator tube (BACTEC-MGIT960) liquid culture and first and second line drug resistance pattern by LINE PROBE ASSAY method.

Method:

Our study was a cross-sectional study carried out from 110 CSF samples aged above 18 years received in TB & DST Lab, Department of Microbiology, IDI block, Kamla Nehru Chest, Hospital, Dr S.N. Medical college Jodhpur, Rajasthan during August 2022 to January 2023. Ethical approval for this study was taken from the ethical committee of DR. SN Medical college Jodhpur. CSF sample from suspected tuberculous meningitis cases who were Aged above 18 YEARS were included. PLHA patients, Samples which got leaked and samples who were aged below 18 years were ruled out. Smear was prepared from CSF samples and directly stained by Ziehl-Neelsen or Auramine O Fluorescent staining, and microscopically examined. From each suspect, the specimen with the highest smear grading based on RNTCP guidelines were tested by LPA. Novel technologies for rapid detection of anti-TB drug resistance have therefore become a priority in TB research and development, and molecular line probe assays focused on rapid detection of rifampicin resistance(alone or in combination with isoniazid)are most advanced.

Result

Male patients were 73.64% and female were 26.36%. Age group 41-50 yrs old patients were highest in number followed by >61 yrs old.

Table 1: Sociodemographic

Age(years)	No. of patients	Percentage
20-30	11	10.00
31-40	25	22.73
41-50	29	26.36
51-60	21	19.09
≥61	24	21.82
Gender		
Male	81	73.64
Female	29	26.36

Nature of CSF samples where 65.45% (72 patients) of CSF samples were clear in nature and 18.18% are turbid followed by 15.45% are blood tinged followed by 0.91% are viscid in nature.

Table 2: Nature of sample

Nature of sample	No. of patients	Percentage
Clear	72	65.45
Bloodtinged	17	15.45
Turbid	20	18.18
Viscid	1	0.91

Out of 110 csf samples only 4.5% are ZN staining positive samples. MGIT culture positive sample 23.64% and 76.36% are MGIT culture negative. MTP64 card test results shows that prevalence of TB in CSF samples is 9.1%.

Table 3: Staining and Culture

	No. of patients	Percentage
MGIT culture Positive	26	23.64
MTP64 card test positive	10	9.1
ZN stain positive	5	4.5

Samples that are turbid in nature, 60% BACTEC MGIT960 culture blood tinged samples are 29.4% positive and have a significant p value 0.0005.
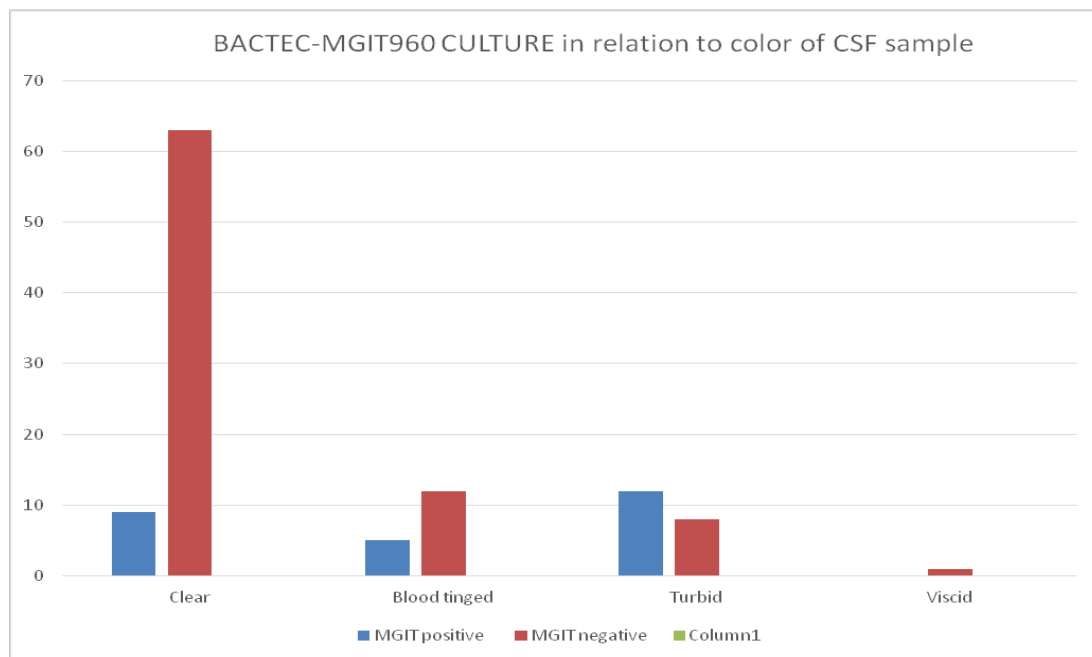

Figure 1: BACTEC and Colour of sample

ZN stain was positive in only 5 samples giving a sensitivity of 50% and a specificity of 100%. ZT stain results in relation to MTP64 results were found to be statistically significant (p<0.0001). MGIT was positive in 26 samples, however only 10 were confirmed to be TB by MTP 64. This gives a sensitivity of 100% and a specificity of 84%. MGIT results in relation to MTP64 results were found to be statistically significant (p<0.0001)

Table 4: ZN staining and MTP64 card test

ZN stain results	MTP64 results				P value
	Positive		Negative		
	N	%	N	%	
Positive	5	50	0	0	<0.0001*
Negative	5	50	100	100	
MGIT					
Positive	10	100	16	16	<0.0001*
Negative	0	0	84	84	

Total samples,which are processed for MTBDRplus genotype LPA,all are sensitive to first line ATT

Table 5: MTBDR Plus Genotype LPA

MTBDR plus LPA	No. of patients	Percentage
Sensitive	10	100.00
Resistance	0	0.00
Total	10	100.00

Discussion

In present study, out of total number of 110 CSF samples 81were male patients samples which accounted 73.64% of total CSF samples and 29 were female patients samples which were 26.36% of total samples .similar observations were seen in Phalguni Malhotra et al,[4] and Krishnapriya Krishnakumariamma[5]

In present study, majority of the samples belongs to the age group (41-50) 26.36%, this was similar to study of Krishnapriya Krishnakumariamma et al in 2020[5] where the mean age group was 31-60yrs.

In the current study, out of total 110 CSF samples, MGIT 960 culture positive samples were 26,which accounts 23.64% and 84 (76.36% samples were MGIT culture negative sample, this was similar to study of Krishnapriya Krishnakumariamma et al.[5] in 2020, where out of 293 CSF samples 22 samples (7.5%) were MGIT culture positive and 271(92.5%)samples were negative.

In current study, out of 26 MGIT 960 culture positive samples, 10 samples (38.4%)were MTP 64 card test positive and 16 (61.5%) were negative for MTP 64 card test. This type result were observed in a study by Vijay GS kumar[6].

In current study MTP 64 card test positive samples were processed for MTBDR plusline probe assay, out of 10 positive samples 10 samples were sensitive for first line anti tubercular drugs. Similar results found in a study by Manke et al 2017[7], in this study 20 samples were processed for first line drug resistance pattern, all samples were sensitive to first line drugs.

In this study ZN stain was positive in only 5 samples giving a sensitivity of 50% and a specificity of 100%. ZT stain results in relation to MTP64 results were found to be statistically significant, this similar result was seen in a study by A. Dorothee Heemskerk2018 et al[8]. similar observation were seen in a study by Ping chen et al 2012[9].

Conclusion

This study reveals that all the samples which are MTP 64 card positive are sensitive to first line ATT.

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