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Original Research Article

A clinical study of tubercular meningitis with special emphasis on CSF PCR as a diagnostic tool

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Abstract

The detection of mycobacterium tuberculosis (M.TB) DNA in the cerebrospinal fluid(CSF) through the use of various molecular methods, particularly polymerase chain reaction (PCR)assay, has emerged as a promising new method for the diagnosis of CNS tuberculosis because of its rapidity, sensitivity, and specificity.

Aims: To study the clinical profile & the role of CSF PCR as an early diagnostic tool in TB meningitis.

Materials and Methods: After clinical evaluation and preliminary tests, patients are separated into two groups, Tubercular meningitis & Non-tubercular meningitis. A technique, polymerase chain reaction (PCR) is used to create enough copies of a particular area of DNA to be tested on CSF.

Results: In the study, 30 individuals had tuberculous meningitis, 13 had pyogenic meningitis, 26 had viral meningitis and 1 had fungal meningitis. TB meningitis patients ranged in age from 18 to 75, with a mean age of 41.8. Mantoux test findings were positive in 86.7% of TBM, 38.5% of pyogenic, and 42.3% of viral meningitis patients. 6.7% of TBM patients had HIV, 7.7% of viral meningitis patients did, but none of pyogenic meningitis patients did. Every patient with TBM, 92.3% with pyogenic, and 53.8% with viral meningitis had elevated CSF Protein. TBM trumps viral and pyogenic. In the TBM group, 83.3% of patients had high ADA levels, 3.8% had viral meningitis, and all pyogenic meningitis patients had normal ADA levels. Gold standard CSF TB PCR and clinical diagnosis, with Sensitivity and specificity of CSF TB PCR are 56.67% and 100%. The sensitivity and specificity of ADA are 83.3 and 97.5%.

Conclusion: PCR alone should not be used as a rationale for starting or stopping the therapy because false negative findings on PCR are sometimes reported. In order to assist clinicians in selecting the best course of treatment, whenever practical, it should be supported by clinical, radiographic, cytological, and other microbiological results (smear microscopy and culturing by traditional and automated technologies).

Keywords: Clinical profile of TB, PCR, meningitis, viral, bacterial

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Introduction

Because of its speed, sensitivity, and specificity, the detection of M.TB DNA in the cerebrospinal fluid using a variety of molecular techniques, in particular the polymerase chain reaction (PCR) assay and nucleic acid amplification (NAA) assay technique, has recently gained attention as a promising new approach for the diagnosis of CNS tuberculosis. Numerous researchers have discussed the value of the PCR assay for finding M.Tb DNA in CSF, despite the fact that there are large variations in the sensitivity of the assay (65-83%) depending on the measuring technique and the facility. Additionally, nested PCR assay has been noted as a popular technique for finding M.Tb DNA in CSF. The sensitivity and specificity of DNA amplification are significantly improved by this novel technique.[1] The goal of the current study is to analyse the clinical profile of tubercular meningitis and the value of CSF PCR in the diagnosis of tb meningitis in light of the variety of clinical presentations and the current diagnostic conundrum.

Method and Materials

Study setting: From 2018 to 2022, this study was conducted at the tertiary care centre. Seventy meningitis patients who were hospitalised throughout the study period and met the inclusion criteria were chosen as study subjects.
This study is both descriptive and case-control. All patients older than 18 years who present with fever and meningeal irritation Any patient on ATT medications, as well as anyone with an intracranial bleed or subdural hematoma, are excluded.

Materials & Methods

Patients who met the inclusion and exclusion criteria and were admitted to our Hospital throughout the study period were included in the study. After receiving signed and informed consent, patients are treated to a thorough clinical examination, history taking, and any necessary investigations. The results are then entered in a pretested proforma.
After thorough clinical evaluation and preliminary test results, patients are separated into two groups.

1) Tubercular meningitis.
2) Non-tubercular meningitis.

Pyogenic meningitis, Viral meningitis, in addition, fungus meningitis on the basis of the criteria listed below, TBM and non-TBM diagnoses were made.

Patient groups

1. Tuberculous meningitis (TBM) patients (n = 27)

a) Subacute or persistent fever with meningeal irritation symptoms include headache, stiff neck, and vomiting, together with or without other CNS involvement symptoms.
b) CSF samples with elevated protein levels, reduced blood sugar (CSF to blood glucose ratio 0.5), and/or pleocytosis with a predominance of lymphocytic cells (100 to 500 cells).
c) MRI or CT findings indicative of tuberculosis
d) Positive ADA

2. Non-TBM patients

a) Pyogenic meningitis: Acute Fever and/or Meningeal Irritation b: Increased Proteins, Decreased Glucose (CSF: Blood Glucose Ratio 0.2) and/or Pleocytosis with

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Predominance of Polymorphonuclear Cells in CSF observations.

b) Viral meningitis patients: Suspected patients in this group comprised those with the following observations: 1) Acute onset of fever and symptoms and signs of meningeal irritation. 2) CSF samples exhibiting a pleocytosis that is primarily lymphocytic, a slight or no rise in protein and frequently normal glucose levels. (Cells 10-100).
c) No extracranial TB clinical evidence.

The ensuing investigations are carried out. Complete hemogram, chest x-ray, Mantoux test, CSF glucose, protein, and chloride, CSF cell type and cell count, CSF ADA analysis, CSF gram stain, AFB, culture, and sensitivity, CSF for TB PCR, CT or MRI brain, and HIV by TriDot. A technique, polymerase chain reaction (PCR) is used to create enough copies of a particular area of DNA to be tested.

Mycobacterium species can be divided into groups according on how pathogenic they may be to humans. Only Mycobacterium tuberculosis and Mycobacterium bovis are covered by the terms tubercle bacillus, Mycobacterium tuberculosis complex (MTC) and references to the illness tuberculosis. Mycobacteria other than Mycobacterium tuberculosis are referred to as "non-tuberculous mycobacteria" or NTM.

Results

70 patients in total, 30 with tuberculous meningitis, 13 with pyogenic meningitis, 26 with viral meningitis and 1 with fungal meningitis are included in study. Patients with TB meningitis ranged in age from 18 to 75, with a mean age of 41.8 years; patients with pyogenic meningitis ranged in age from 18 to 76, with a mean age of 38.0 years; and patients with viral meningitis ranged in age from 20 to 75, with a mean age of 46.3 years. 50% men and 50% women made up the TBM group. In the group with pyogenic meningitis, men made up 76.9% and women, 23.1%. Again, there were 50% males and 50% females in the group with viral meningitis. Each patient in each of the three groups had a fever. Patients with TB meningitis experienced headaches in 86.7% of cases, while those with pyogenic and viral meningitis had headaches in 100% of cases. In the TBM group, 73.3% of patients had altered sensorium at the time of presentation, compared to 69.2% of patients with pyogenic meningitis and 73.1% of patients with viral meningitis. Seizures were experienced by 30% of patients in the TBM group, 46.2% of patients with pyogenic meningitis and 38.5% of patients with viral meningitis. In the TBM group, 33.3% of patients had papilloedema, compared to 23.1% in the pyogenic meningitis group and 11.5% in the viral meningitis group. All of the patients in the three groups had stiff necks. While they were not seen in cases of pyogenic and viral meningitis, 30% of patients in the TBM group had cranial nerve palsies. Hemiplegia was seen in 16.7% of TBM patients but not in pyogenic or viral meningitis patients. Therefore, only the TBM group showed neurological impairments. None of the patients with viral meningitis were comatose, but 10% of those with TBM and 7.7% of those with pyogenic meningitis were.

Table 1: Clinical presentation of different meningitis Groups

Signs and symptoms	Group						
	TB Meningitis		Pyogenic Meningitis		Viral Meningitis		
	n	%	n	%	n	%	P
Fever	30	100.0	13	100.0	26	100.0	NA

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Headache	26	86.7	13	100.0	26	100.0	0.06
Altered Sensorium	22	73.3	9	69.2	19	73.1	0.9
Seizures	9	30.0	6	46.2	10	38.5	0.6
Papilloedema	10	33.3	3	23.1	3	11.5	0.4
Neck_Stifness	30	100.0	13	100.0	26	100.0	NA
Cranial_Nerve_Palsies	9	30.0	0	.0	0	.0	0.001
Hemiplegia	5	16.7	0	.0	0	.0	0.03
Coma	3	10.0	1	7.7	0	.0	0.3

In the TBM group, 43.3% of patients, 100% of patients with pyogenic meningitis, and 30.8% of patients with viral meningitis had higher total counts.
3.8% of the individuals in the group with viral meningitis had it lessened.
13.3% of patients in the TBM group had thrombocytopenia, compared to 53.8% of patients with viral meningitis and none with pyogenic meningitis. Those with viral meningitis exhibited thrombocytopenia most frequently.
Creatinine levels were raised in 10% of TBM patients, 23.1% of pyogenic meningitis patients, and 15.4% of viral meningitis patients. Mean ESR values for the TBM group were 66, 38.15 for pyogenic meningitis and 43.08 for viral meningitis. In comparison to pyogenic and viral meningitis, TBM group exhibits greater ESR. Mantoux test results were positive in 86.7% of TBM patients, 38.5% of pyogenic meningitis patients and 42.3% of viral meningitis patients. HIV was present in 6.7% of patients with TBM, 7.7% of patients with viral meningitis, but not in any of the patients with pyogenic meningitis.
CSF blood glucose ratio was <0.6, CSF Glucose was lowered in 86.7% of TBM patients, 100% of pyogenic meningitis patients, and 15.4% of viral meningitis patients. The CSF glucose level is typically unaffected in the viral meningitis group. Viral vs. TBM is important. CSF Protein was raised in every patient in the TBM group, 92.3% in the group with pyogenic meningitis, and 53.8% in the group with viral meningitis. TBM is much greater than viral and pyogenic. In the TBM group, 83.3% of the patients had increased ADA levels, 3.8% had viral meningitis, and all the patients in the pyogenic meningitis group had normal levels. The pyogenic group had a high mean cell count. Lymphocytic preponderance in the other two groups, with neutrophilic predominance in the pyogenic group. When compared to the viral meningitis group, the TBM group's cell count was noticeably higher. 56.7% of the patients in the TBM group had good results, while none of the patients in the other two groups did. In contrast to the other two groups, 16.7% of TBM patients had basal exudates and infarcts. 16.7% of TBM patients had hydrocephalus, compared to 7.7% of pyogenic meningitis patients and viral meningitis patients who did not have it. 26.7% of TBM patients, 23% of patients with pyogenic meningitis, and 15.3% of patients with viral meningitis all had cerebral oedema. 6.7% of TBM patients had tuberculoma or a tubercular abscess. Validity of the CSF TB PCR and clinical diagnosis as the gold standard. Table 2 describes the number of cases positive for CSF TB PCR in TB, pyogenic and viral meningitis. The sensitivity and specificity of CSF TB PCR are 56.67% and 100%, respectively (Table 3) in the present study. The sensitivity and specificity of ADA are 83.3% and 97.5%, respectively in then present study (Table 4).

Table 2: CSF TB PCR in different groups of meningitis

		Group					
		TB Meningitis		**Pyogenic Meningitis**		**Viral Meningitis**	
		Count	**Column N %**	**Count**	**Column N %**	**Count**	**Column N %**
CSF_TBPCR	Positive	13	43.3%	13	100.0%	26	100.0%
	Negative	17	56.7%	0	.0%	0	.0%

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Table 3: The sensitivity and specificity of CSF TB PCR IN TBM

Parameter	Estimate	Lower-Upper 95% CIs
Sensitivity	56.67%	(39.2, 72.62)
Specificity	100%	(91.03, 100)
Positive Predictive Value	100%	(81.57, 100)
Negative Predictive Value	75%	(61.79, 84.77)
Diagnostic Accuracy	81.16%	(70.39, 88.65)
Likelihood ratio of a Positive Test	'undefined'	('?'-'undefined')
Likelihood ratio of a Negative Test	0.4333	(0.3727-0.5038)
Cohen's kappa (Unweighted)	0.5965	(0.3806-0.8124)

Table 4: The sensitivity and specificity of ADA in TB PCR of TBM

Parameter	Estimate	Lower-Upper 95% CIs
Sensitivity	83.33%	(66.44, 92.66)
Specificity	97.5%	(87.12, 99.56)
Positive Predictive Value	96.15%	(81.11, 99.32)
Negative Predictive Value	88.64%	(76.02, 95.05)
Diagnostic Accuracy	91.43%	(82.53, 96.01)
Likelihood ratio of a Positive Test	33.33	(4.622-240.4)
Likelihood ratio of a Negative Test	0.1709	(0.1154-0.2533)

Discussion

TBM's non-specific symptoms make its clinical presentation difficult to identify from that of other meningitis forms. The study involved 70 patients in total, 30 of them had TB meningitis, 13 had pyogenic meningitis, 26 had viral meningitis, and one had fungal meningitis. With a mean age of 41.8 years, TB meningitis affected people between the ages of 18 and 75. Male to female ratio in the TB meningitis group was 1:1.

Fever was the most frequent symptom, followed by headaches, sensorium changes, and seizures.

Neck stiffness affected all of the patients. 33.3% of the patients had papilledema, 30% had cranial nerve palsies, 16.7% had hemiplegia and 10% were in a vegetative state. Only TB meningitis patients had cranial nerve palsies, including oculomotor nerve palsies and hemiplegia, which were not present in individuals with other types of meningitis.

Thrombocytopenia was found in 53.8% of patients with viral meningitis and 13.3% of patients with TB meningitis, but not in patients with pyogenic meningitis. Compared to other categories, patients with TB meningitis had a lower prevalence of renal failure. The Mantoux test was positive in 86.7% of patients with TB meningitis, but it was also positive in 38.5% of patients with pyogenic meningitis and 42.3% of patients with viral meningitis, so it is not a reliable test. Mantoux can, however, be positive in patients who have latent infections or a history of BCG immunisation. When compared to other groups, TB meningitis patients' mean ESR was high. It must be emphasised that HIV testing should always be done in conjunction with detecting TBM because active TB is more likely in those living with HIV. Only two patients (6.7%) in the TB meningitis group and 7.7% of the patients in the viral meningitis group in our study tested positive for HIV. All of the patients in the group with pyogenic meningitis had negative results. 13.3% of patients in the TBM group had coexisting pulmonary TB, however none of the patients in the pyogenic or viral meningitis groups had extrapulmonary or pulmonary tuberculosis.

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One of the frequent clinical signs of extra-pulmonary tuberculosis is TBM. In the past 20 years, TBM has become more popular in developing nations like India. Due to TBM's pleomorphic clinical appearance, changeable CSF cellular content, and biochemical characteristics, which are comparable to cases of partially treated pyogenic meningitis, the diagnosis is challenging to confirm. Many significant CNS problems may be linked to delayed diagnosis and treatment. Therefore, early M. tuberculosis detection is crucial for the accurate diagnosis and treatment of tuberculous meningitis. The crucial technology of CSF analysis provides evidence that suggests TB meningitis. In our study, CSF glucose was lowered in 86.7% of patients with TB meningitis, 100% of patients with pyogenic meningitis, and 15.4% of patients with viral meningitis. This supports the idea that in viral meningitis, CSF glucose is frequently normal. Mean CSF protein levels were 65.6 mg/dl in the viral meningitis group, 158.7 mg/dl in the TBM group, and 121.9 mg/dl in the pyogenic meningitis group. It was noticeably higher in the group with TB meningitis. The mean total CSF cell count was 225 in the lymphocytic-predominant TB meningitis group, 348 in the neutrophil-predominant pyogenic meningitis group, and 36 in the lymphocytic-predominant viral meningitis group.

In all of the patients, the CSF for AFB was negative. The paucibacillary character of CSF in the current investigation can be used to explain the limited sensitivity of the AFB microscopy. For an AFB microscopy result to be considered positive, the specimen must contain a significant amount of bacteria (>104-105 bacilli/ml) [1]. Numerous investigations found that the smear microscopy for TBM had similar sensitivities that ranged from 0 to 20%. Another diagnostic indicator for TB meningitis is the presence of levels of ADA in CSF. In addition to being quick, easy, and affordable, ADA estimate in CSF is also a fairly specific method for determining the cause of tuberculosis in cases of tuberculosis meningitis [2, 3]. Because it can be found in bodily fluids such pleural, pericardial, and peritoneal fluid, ADA is a helpful surrogate sign for TBM [5]. Because mycobacterial antigens stimulate T lymphocytes, the levels of ADA rise in TB. Macrophages are the white blood cells that initially take in the tuberculosis bacteria; they then digest and destroy the foreign particles by taking them into a vacuole. Despite being ingested, the bacteria prevents the cell from breaking it down after it has entered. This indicates that a tuberculosis bacteria has now been transmitted to the WBCs. The TB bacterium needs to attract additional WBCs to the area in order to produce the granuloma. The easiest approach to do this is to rupture the cell that it is currently in, as the immune system will be triggered by the fragments of broken-up cell and will produce granulomas as a result. Adenosine levels rise as a result of granuloma formation, and as adenosine levels rise, so does the activity of the enzyme adenosine deaminase. The catabolic enzyme adenosine deaminase is crucial to the purine salvage pathway. The transformation of adenosine into adenosine and ammonia is carried out by this enzyme. There are elevated ADA levels in several types of tuberculosis. So, it serves as a marker for tuberculosis. However, ADA activity is heightened in a number of other conditions, including malignant tumours, typhoid, viral hepatitis, mononucleosis and early stages of AIDS. As a result, there is no documented instance of positive tuberculosis meningitis from the ADA activity. However, if TB PCR is performed afterward, it may produce positive findings. Therefore, TB PCR should be used to monitor ADA activity. In the current study, the ADA has a sensitivity and specificity of 83.3% and 97.5%, respectively. In several trials, the ADA's sensitivity ranged from 44 to 100%.

TBM diagnosis has been a constant struggle. Although mycobacterium detection by microscopy and culture is still the gold standard for tuberculosis diagnosis, mixed infections and limited sensitivity in detecting low microbe concentrations in CSF present substantial challenges. As a result, the diagnosis is still largely predicated on clinical symptoms, neurologic signs, CSF results, CT scans, and the patient's reaction to anti-TB medications [5]. Despite many important improvements in diagnostic procedures, diagnosis nevertheless

frequently remains a challenge. A timely diagnosis is critical to the effectiveness of the treatment. The detection of microbial disorders has significantly advanced thanks to the CSF PCR assay and TBM is no exception. Due to its rapidity, the identification of Mycobacterium tuberculosis DNA via PCR is a common diagnostic technique. To achieve high specificity, it's crucial to choose Mycobacterium tuberculosis-specific DNA carefully and to avoid contaminating CSF samples. Two studies using polymerase chain reactions on TBM patients found that the sensitivities and specificities varied greatly. The vast majority of studies indicated modest sensitivity (about 50%) but good specificity (up to 98%) for this test in the diagnosis of TBM. There have been reports with sensitivity ranging from 35 to 98% and 100% specificity [6, 7].

In our investigation, Nested PCR has a sensitivity of 56.6% and a specificity of 100%.

There may be a variety of causes for the lower sensitivity. First, there are PCR inhibitors present, which are discovered to be more frequently associated with extrapulmonary specimens than pulmonary ones. Due to the intricacy of the cell wall, mycobacteria do not lyse well during the extraction process [8, 9]. Thirdly, the IS6110 sequence is missing from several Asian M. tuberculosis strains [5]. Therefore, if the mycobacteria present in these samples lacked the IS6110 sequence, some of the instances in the current investigation might not have been discovered [10, 11]. The dispersion of the microbes was not uniform, which was the fourth factor. Fifth, the sample's incapacity to concentrate (due to less volume). Due to the limited quantity of bacilli in the sample, the volume of the sample is crucial for PCR, particularly in TBM. Sixth, the sample processing method has an impact on the sensitivity.

Limitations: Our study has a few significant limitations. First, a small number of cases were evaluated due to the short study duration. Second, a sizable sample of CSF is required for TB detection using an acid-fast bacilli (AFB) staining smear and PCR, but due to our patient's conditions, this was not possible. Third, we skipped the TB culture test because it takes 6–8 weeks for a positive result and has low sensitivity. Fourth, our PCR standard did not include amplification.

Conclusion

TBM is a deceptive illness, but it can also manifest itself in an unusual way as acute or subacute meningitis. As a result of the unique clinical presentation and the paucibacillary character of the sample, TBM is frequently difficult to diagnose. Comparing nested PCR to smear microscopy, it was discovered to be more sensitive. Nested PCR, in our opinion, merits a spot in the laboratory diagnosis of TBM, but strict adherence to the test protocol is required. PCR alone should not be used as a rationale for starting or stopping the therapy because false negative findings on PCR are sometimes reported. In order to assist clinicians in selecting the best course of treatment, whenever practical, it should be supported by clinical, radiographic, cytological, and other microbiological results (smear microscopy and culturing by traditional and automated technologies).

References

1. Takahashi T, Tamura M, Takasu T. The PCR-Based Diagnosis of Central Nervous System Tuberculosis: Up to Date, 2012, 1-17. https://doi.org/10.1155/2012/831292.
2. Deshpande PS, Kashyap RS, Ramteke SS, Nagdev KJ, Purohit HJ, Taori GM, *et al*. Evaluation of the IS6110 PCR assay for the rapid diagnosis of tuberculous meningitis. Cerebrospinal Fluid Res. 2007;4:10. Doi: 10.1186/1743-8454-4-10. PMID: 17976247; PMCID: PMC2206054.
3. Marx GE, Chan ED. Tuberculous meningitis: diagnosis and treatment overview. Tuberc

Res Treat., 2011, 798-764. Doi: 10.1155/2011/798764. PMID: 22567269; PMCID: PMC3335590.

4. Mastroianni CM, Paoletti F, Lichtner M, D'Agostino C, Vullo V, Delia S. Cerebrospinal fluid cytokines in patients with tuberculous meningitis. Clin Immunol Immunopathol. 1997;84:171-6. Doi: 10.1006/clin.1997.4367. PMID: 9245549.
5. Blacklock JWS, Griffin MA. Tuberculosis meningitis: problems in pathogenesis and treatment. J Pathol. Bacteriol. 1935;40:489-502.
6. Caws M, Wilson SM, Clough C, Drobniewski F. Role of IS6110-targeted PCR, culture, biochemical, clinical and immunological criteria for diagnosis of tuberculous meningitis. J Clin Microbiol. 2000;38:3150-5. Doi: 10.1128/JCM.38.9.3150-3155.2000. PMID: 10970348; PMCID: PMC87341.
7. Bhigjee AI, Padayachee R, Paruk H, Hallwirth-Pillay KD, Marais S, Connoly C. Diagnosis of tuberculous meningitis: clinical and laboratory parameters. Int J Infect Dis. 2007;11:348-54. Doi: 10.1016/j.ijid.2006.07.007. PMID: 17321183.
8. Horne NW. Tuberculous meningitis: problems in pathogenesis and treatment. Edinb Med J. 1951;58:413-29. PMID: 14872801; PMCID: PMC5288073.
9. Inhibition of cytokines expression in human microglia infected by virulent and non-virulent mycobacteria. International Journal of Leprosy and Other Mycobacterial Diseases. 2004;72:199.
10. Rock RB, Olin M, Baker CA, Molitor TW, Peterson PK. Central nervous system tuberculosis: pathogenesis and clinical aspects. Clin Microbiol Rev. Table of contents. 2008;21:243-61. Doi: 10.1128/CMR.00042-07. PMID: 18400795; PMCID: PMC2292571.
11. Farer LS, Lowell AM, Meador MP. Extrapulmonary tuberculosis in the United States. Am J Epidemiol. 1979;109:205-17. Doi: 10.1093/oxfordjournals.aje.a112675. PMID: 425959.