ORIGINAL ARTICLE

THE ROLE OF CSF PCR IN EARLY DIAGNOSIS OF TUBERCULAR MENINGITIS: A STUDY FROM BIDAR DISTRICT

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ABSTRACT: OBJECTIVES: The objective of this study was a study the clinical features and laboratory profile including acid fast bacillus microscopy, cerebrospinal fluid (CSF) culture and M tuberculosis polymerase chain reaction (PCR) for an early diagnosis of tuberculous meningitis. **METHODOLOGY**: 40 consecutive patients, fulfilling the clinical criteria of tuberculosis meningitis, were included in the study. All patients were subjected to detailed clinical and laboratory evaluation and radiological tests. CSF acid fast bacillus microscopy culture and PCR were carried out in all and results were analyzed. **RESULTS:** Majority of the patients presented with fever headache, vomiting and signs of meningeal irritation. Ziehl –Neelsen staining for acid fast bacillus was positive in 4% cases, culture in 18% cases and CSF PCR for mycobacterium tuberculosis was positive in 68%. Milliary pattern on chest x-ray was seen in 16% and CNS tuberculomas were seen in 14% cases. **CONCLUSION:** The CSF PCR estimation in clinically suspected cases of tuberculous meningitis may be an extremely valuable test for early diagnosis and institution of specific therapy.

KEYWORDS: Arachnoiditis, Miliary Tuberculosis, polymerase chain reaction, tuberculoma, tuberculous meningitis.

INTRODUCTION: Tuberculous meningitis (TBM) continues to be an important cause of morbidity and mortality throughout the world.[1] i constitutes 7-12% of all cases of tuberculosis and has varied clinical presentation.[2,3] Very few studies with a comprehensive evaluation of the clinical and diagnostic aspects of tuberculosis meningitis are available from India.[4-8] the present study was conducted with the objective to analyze clinical features and laboratory tests like acid-fast bacillus (AFB) microscopy, cerebrospinal fluid (CSF) culture and especially the sue of CSF M tuberculosis polymerase chain reaction (PCR) for an early diagnosis of tuberculous meningitis (TBM). Rapid and accurate diagnosis would in turn therapy with a consequent reduction in morbidity and mortality and would also allow more specific rather than empirical use.

MATERIAL AND METHOD: 40 consecutive patients who fulfilled the clinical criteria of tuberculous meningitis, admitted in the department of neurology, BRIMS Teaching Hospital, Bidar during the period of one year from Jan-2012 to Dec-2012, were included in the study.

Patients presenting with a clinical picture suggestive of chronic meningitis like fever (fore more than 2 weeks) headache, vomiting, signs of meningeal irritation with or without altered mental status and fulfilling the following definite or probable criteria for diagnosis of TBM were included in the study. The definite criteria included a positive culture or demonstration of

Mycobacterium tuberculosis by Ziehl-Neelsen (ZN) stain, while the probable criteria comprised CSF lymphocytic pleocytosis (>10 cells mm^3), increased protein concentration (>40 mg/dl) and sugar concentration less than 60% of corresponding blood glucose, imaging suggestive of chronic meningitis including basal exudates, hydrocephalus, meningeal enhancement, granulomas and infracts, history of contact with a patient of tuberculosis or evidence of tuberculosis elsewhere in the body. 16 patients who either had CSF typical for pyogenic / fungal meningitis, with a positive Gram-staining or culture for other organisms, with response to antimicrobial treatment or had imaging suggestive of subarachnoid haemorrhage were excluded and were taken as the negative control group.

According to the classification by Gordon and Parsons[9] patients were classified into three clinical stage, stage I; patients fully conscious and oriented with signs of meningeal irritation but no focal signs and no evidence of hydrocephalus, stage II; patients who were confused and / or had focal neurological deficits and sage III; patients who were stuporous, delirious or comatose with hemiplegia or paraplegia or other neurological deficits.[10]

Processing of samples for microbiological tests: All the samples of CSF were collected in gamma irradiated disposable plastic containers. About 1-3ml of CSF was centrifuged at 13,000 rpm for 20 minutes. The supernatant was discarded, about 300µl was retained with the pellet.

AFB staining with ZN satin: 20 µl of this was used for preparation of AFB smear, stained by ZN method as per RNTCP guidelines.[11] All CSF smears were stained by ZN method for AFB using strong carbol fuschin acid alcohol as decolorant and methylene blue as the counter stain After staining, more examined by the light microscope using oil immersion (x100) lens.

Mycobacterium tuberculosis culture: 150 µl was inoculated onto 2 slants of Lowenstein Jensen (L J) media and incubated at 37°C. All culture samples were examined once a week for 8 weeks. The positivity of culture was defined by the growth of Mycobacteria on L J media.

Positive culture was tested by selective biochemical tests for the definitive identification of M tuberculosis.

CSF polymerase chain reaction: 100 µl of sample was used for DNA extraction by physiochemical method (Van Madden et al 1993, Van Soligen et al 1995)[12,13] and rest of the sample was stored at – 20° for future use.

CSF polymerase chain reaction was done in all cases using single tube nested PCR (in house method).[14] two sets of primers and probes from the mycobacterium tuberculosis genome, encoding the insertion sequence IS 6110 and the 38kilodalton protein were used. These sets of primers and the probe were used initially to screen all specimens. If the specimen was negative with the IS 6110 assay, it was retested in the 38kilodalton assay because of all the M tuberculosis isolates contained the 38kilodalton sequence and as some Indian strains may not have IS 6110 or have low copy number of IS6110.

A concurrent assay was also carried out with the IS6110 primers spiking with 2pg M tuberculosis DNA to detect inhibitors.

PCR conditions – The total reaction volume was 100 µl and contained the following: 10mM tri-HCL (pH 8.3) (Sigma, St.lousi, Missouri, USA); 50mM KCI; 2mM $MgCl_2$: 0.15mM dATP, dGTP, dCTP (Pharmacia Biotechnology, Uppsala, Sweden), and dUTP (Boehringer Mannheim, Mannheim, Germany); 2pmol external primers; 75pmol internal primers; 2 units taq polymerase (US Biochemical Corp., Cleveland, ohio, USA); 0.5 units Uralic-N-glycosidase (Boehringer Mannheim).

The mixture was incubated at 37°C for 10 minutes to inactivate this enzyme. The PCR condition were as follows: 94°C for 45 seconds and 72°C for 1.5 minutes for the first 15 cycles for both annealing and extension in view of the higher melting temperature of the external primers (88°C) and the 94°C for 45 seconds; 55°C for 45 seconds and 72°C for one minute for 45 cycles. These PCR condition were used for both assays. A stringent annealing temperature for the external primers is essential for the production of specific ampicons for the next 45 cycles. During re-implication, a much lower annealing temperature was used for greater efficiency rather than for stringency.

Positive and negative controls were included in each run and precautions against cross contamination were taken. Contrast enhanced cranial computerized tomography (CT) scan were done using a third generation, CT scanner. MRI brain / spine was done on 1.5 T using T1, T2, FLAIR and gadolinium T1 sequence and the presence of hydrocephalus, exudates, infarctions and tuberculomas were noted. Evidence of tuberculosis elsewhere in body was screen by relevant investigations.

All the patients were screened for HIV using HIV enzyme-linked immunosorbent assay (ELISA) and western blot was carried out in ELISA positive cases.

OBSERVATIONS: Out of 40 patients included in the study there were 23 females and 17 males. The mean age of patients was 32 years (range 11-80 years). The highest age specific incidence was seen in the age group 11-30 years. The major clinical presentations were fever (n=48), headache (n=42), vomiting (n=45), neck rigidity (n=44) altered sensorium (n=29), seizures (n=11), focal neurological deficits (n=12), cranial nerve palsies (n=13) and papilloedema (n=17). The mean duration of illness was seen to 34days, past history of tuberculosis was present in 7 cases wile history of contact with tuberculosis was noted in 2 cases.

CSF findings	Mean values
Total leukocyte count	69.52 cells/ml
Glucose	36.22 mg/dl
Proteins	136.18 mg/dl

Table 1: Showing Mean CSF Finding in Patients of TBM

Category	AFB microscopy with ZN stain	Culture on LJ Media	CSF PCR	No. of Cases (n=40)
Definite TBM (n=9)	+	+	+	2
	-	+	+	5
Possible TBM (n=41)	-	-	+	21
	-	-	-	12
Negative control (n=20)	-	-	+	1
	-	-	-	15
Sensitivity	2/40 (4%)	9/40(18%)	34/40 (68%)	
Specificity	20/20 (100%)	20/20 (100%)	19/20(95%)	

Table 2: Showing Distribution of Cases according to CSF PCR Culture and Microscopy Findings in Patients of TBM

Majority of patients presented in stage 1 and 2 of the disease and only 14% cases were seen in the stage of 3 tuberculosis meningitis. CSF was abnormal in all and findings are listed in Table 1 and Table 2. The CSF cell counts ranged from 10-450 cells / mm^3 (mean 65.92/mm^3) with more than 90% lymphocytes, proteins ranged from 48-470 mg/dl (mean 136.18 mg/dl), sugar ranged from 12 to 68 mg/dl (mean 36.2 mg/dl). CSF was positive fro AFB microscopy in 2 cases (4%), culture in 9 cases (18%) and for PCR in 34 cases (68%). In definite TBM 89% and in probable TBM 6.41% cases showed PCR positivity PCR showed specificity of 95%. HIV serology was positive in 2 patients.

CT/ MRI scan revealed tuberclomas (6 cases), infarcts (8 cases), hydrocephalus (12 cases) and basal exudates (14 cases). Spinal MRI scans were carried out in 4 patients and revealed intramedullry thoracic tuberculoma in 1 and arachnoiditis in 3 patients. Evidence of pulmonary tuberculosis on chest radiography was noted in 22% cases (milliary pattern in 16% and apical lesion in 6% cases).

DISCUSSION: TBM is a medical ailment that required urgent treatment because early intervention reduces the risk of complications. Rapid detection of Mycobacterium tuberculosis is of vital importance for the proper diagnosis and management of TBM. Various methods available for M tuberculosis detection are CSF AFB microscopy, culture takes up to 8 weeks and is also often negative. This study revealed positively of CSF AFB microscopy n 4% culture in 18% cases but the specificities of these methods were found to be 100%. Rapid techniques based nucleic acid amplification such as PCR have been reported to be more sensitive (sensitive ranging between 50-91% in various studies).[15-21]

In this study the definite cases of TBM had PCR positivity in 89% and probable cases had positivity in 63.41% only. The overall sensitivity of CSF PCR observed was 68% and specificity was around 95%. It will therefore be useful to carry out PCR as a proficient technique for rapid diagnosis of TBM. Even though PCR is more expensive, it can specifically identify M tuberculosis in a clinical specimen within 7-8 hours. This high rate of positive results in definite and probable

cases of TBM suggests that PCr should be included as a priority test for confirming TBM in addition to AFB staining or culture positivity.[22] PCR positivity was especially higher in cases associated with tuberculomas (85%), arachnoiditis (66%), military shadows in the chest (87.5%) and HIV positively (100%) which probably correlates with a high bacilli load in these conditions.

It interesting to note that, PCR positivity was in stage I and II was 96.5%, while in stage I the positivity was 23.8%. In view of this observation, repeat PCR testing in patients with early negative tests may be advisable, which may increase the yield of positive results and may help in making a more definitive diagnosis, paving way for specific therapeutic regimens. CNS tuberculomas with tuberculous meningitis have not been commonly reported in literature.[23-25] In this study tuberculomas were seen in 14% cases with TBM which is much more than in other series.[25] tuberculomas were present both supra and infratentorially although more frequently in supratentorial locations[26] and were frequently multiple.

The pathogenesis of tuberculomas is still far from clear. The hypotheses regarding the pathogenesis of tuberculoma formation in the presence of TBM are either hypersensitivity to M tuberculosis protein of coalescence of preexisting tiny tubercles in the leptomeninges as a result of further immunosuppression and consequent increasing load of bacilli. In this series the latter seems to be the more likely explanation as those cases with co-existing TBM and CNS tuberculomas had a high rate of PCR positivity correlating with higher replication of tubercle bacilli in the body. Higher association of tuberculomas in this study might also be attributed to the newer imaging techniques like MRI (1.5T) which are now freely available and were extensively used in this study.

Although spinal tuberculous arachnoiditis is a rare complication of CNS tuberculosis that can results in various neurological deficits and usually occurs several months or years after arrest of disease, it may occur during the acute stage as well, or after variable period, from the onset of TBM.[27-29] There cases of archnoiditis associated with TBM were found who presented as asymmetrical quadriparesis with sensor and bladder involvement at the time of admission. These patients were in middle age with high CSF protein and PCR positivity.

The present study documents HIV infection 4% cases but there were no significant difference in clinical profiles of HIV positive and HIV negative patients; however the imaging showed scant meningeal enhancement and basal exudates in PCR positive HIV positive cases as compared to other PCR positive HIV negative patients.[30, 31] Military pattern the chest radiography was seen in 16% cases.[32, 33] The patients with TBM and military shadows in chest had a younger age at onset, shorter duration of illness, higher diseases stages (I, II) and presence of seizures. CNS infection in these cases occurred as part of disseminated tuberculosis and these patients might have had a highly compromised immune status leading to a rapid and severe manifestation of the disease. Thus patients TBM with military shadows in the chest had a more severe disease course.

Arachnoiditis and tuberculomas are important complications of CNS tuberculosis which may co-exist with tuberculosis meningitis and need to be managed accordingly. This study highlights the values of CSF PCR for M tuberculosis as a good diagnostic aid especially when it is associated with complications like arachnoiditis or tuberculomas. The cerebrospinal fluid PCR estimation in clinically suspected cases of TBM may be extremely valuable in early and accurate

diagnosis and rapid institution of specific therapy which may contribute to reduction in morbidity and mortality.

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