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Introduction

Tuberculosis is a major cause of morbidity and mortality worldwide. The World Health Organization has noted that the global incidence of TB is increasing by 0.4% per annum [1]. Central nervous system TB accounts for about 5% of all extra-pulmonary TB and Tuberculous Meningitis (TBM) is the most serious complication [2]. Delay in the diagnosis and institution of therapy can result in neurological sequelae in 20-25% of cases [3]. Prompt and accurate diagnosis is of paramount importance for better patient outcome. Conventional modalities like microscopy and culture, although considered as gold standard has low sensitivity and limited role in diagnosis of pauci-bacilliary condition like TBM.

The development of rapid, sensitive and specific test for detection of mycobacterium has been a long standing need. A number of mycobacterial antigen [4,5] and antibody kits [6,7] have been developed but are quite inadequate and needs validation.

Nucleic Acid Amplification Techniques (NAAT) such as Polymerase Chain Reaction (PCR) has been reported to be more sensitive and specific. Several *Mycobacterium tuberculosis* specific sequences like IS6110, Protein antigen b [8], MPB64 and 65 kDa have been evaluated [9,10]. Most of the earlier PCR based studies have used IS6110 because of its repetitive nature [9,11]. However, either absence or presence of a few copies of this sequence have already been reported in some isolates [12-15]. Studies from India have also reported that a large number of clinical isolates (11-40%) had either a single copy or no copy of insertion sequence [16,17]. MPB64 has been demonstrated to be highly specific for *Mycobacterium tuberculosis* complex [18,19] and other studies have reported sensitivity and specificity of 75-90% and 100% respectively. The literature regarding the evaluation of MPB64 in patients of TBM in our region is scanty. Therefore, in the present study, we evaluated PCR based assay using MPB64 primers specific for *Mycobacterium tuberculosis* complex in CSF samples of patients with TBM and non TBM control group and its comparison with conventional techniques like microscopy and culture.

Materials and Methods

A total of 130 CSF samples received for AFB smear and culture in laboratory of tertiary care hospital of India, between September 2008 and December 2009 were evaluated. Patient's age ranged from 12-90 years. The relevant history and other details of the patients were noted from the case records. The patients were divided into 3 groups: Group I: TBM (n=90): (a) confirmed TBM-culture/smear positive (n=9), and (b) suspected TBM: smear/culture negative, clinical and laboratory features suggestive of TBM and response to anti-tuberculosis therapy [20] (n=81); Group II: Non-TBM infectious meningitis (n=20): (a) pyogenic meningitis (n=8), (b) viral meningitis (n=10), and (c) fungal meningitis (n=2); Group III: Non-infectious neurological disorders(n=20): head injury (HI: n=10), Landry-Guillain-Barre syndrome (LGBS: n=2), multiple sclerosis (n=2) and tumors (n=6). The present study is a part of project approved by institute ethic committee.

Processing of CSF sample

All the 130 CSF samples were subjected to three microbiological tests: Ziehl-Neelsen staining (ZN), culture on Lowenstein-Jensen medium and PCR with MPB-64. The CSF samples of the subjects were

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this was also positive by culture and MPB64 PCR which emphasize on the paucibacillary nature of TBM. Out of 9 con rmed TBM (culture positive) cases, 8 (88.8 %) were positive by MPB64 PCR. Whereas out of 81 suspected TBM patients, MPB64 PCR was positive in 66 (81.48%) patients (Table 1) and all 66 patients had shown good response to anti-tuberculous drug therapy. e response to treatment was judged by the experienced neurologists in treating TBM. In control group, all 40 patients showed negative result in all the three tests, thus giving 100 percent speci city.

A nal diagnosis of TBM was made in 90 patients, based on results of culture, microscopy, PCR and response to anti tuberculous drug therapy (Table 2). Of these 90 patients, PCR was positive in 74, culture in 9, and microscopy in one. us the sensitivity of MPB64 PCR, culture and microscopy was 82.22%, 10% and 1.11% respectively. However the sensitivity of PCR in the con rmed TBM and suspected TBM group was 88.8% and 81.46% respectively. In non TBM group PCR was negative in all cases. Hence, the speci city was 100%.

Disc ssion

Tuberculous meningitis is one of the most serious manifestations of extra-pulmonary tuberculosis and prompt diagnosis and treatment is required for better clinical outcome. e conventional methods like microscopy and culture are quite insensitive in paucibacilliary conditions. So, the present study was carried out to evaluate the utility of PCR targeting MPB64 gene in comparison with conventional techniques like microscopy and culture for the rapid diagnosis of TBM.

ough microscopy is very economical, it has limitation of low sensitivity in extra-pulmonary paucibacilliary conditions like TBM. In the present study AFB smear was positive in only one patient, which was also positive for culture and PCR. e number of bacilli required for positive acid-fast staining is 10^4/ml. Our results are similar to previous studies, which had shown sensitivity in range of 0-10%la9455-92(w)

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