Evaluation of PCR Using TRC₄ and IS₆₁₁₀ Primers in Detection of Tuberculous Meningitis

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Tuberculosis (TB) is the major cause of death worldwide and is due to a single pathogen (16). Childhood TB in general and TB meningitis in particular owe their existence to unsuspected, undiagnosed, or incompletely treated adults in the community. Among children, mortality due to TB occurs mainly due to the neural form of TB, namely, TB meningitis. About 3% of the pediatric admissions to hospitals in India are due to TB meningitis, with reported mortality ranging from 17 to 71% (2, 14). Prompt diagnosis is critical for initiating appropriate therapy and facilitating measures to prevent dissemination of this highly contagious disease. The prevalence of childhood TB meningitis remains largely underestimated because clinical manifestations are nonspecific in early stages of the disease and bacteriologic confirmation is available only for a small proportion of patients. Also, clinical diagnosis of childhood TB meningitis is difficult due to its varied clinical presentations. Further, routinely used tests employed for clinical diagnosis of TB are inadequate to detect extrapulmonary forms of TB like TB meningitis.

PCR is currently the most sensitive and rapid method to detect extrapulmonary Mycobacterium tuberculosis (1, 5, 8, 11, 15). We used as a new target TRC₄, which was cloned and characterized previously in our laboratory (10). TRC₄ is a conserved repetitive element with specificity for M. tuberculosis complex. The aim of this paper was to compare the efficiency of a PCR with a target chosen from this cloned fragment with that of a PCR with the widely used IS₆₁₁₀ sequence in detecting M. tuberculosis in cerebrospinal fluid (CSF) samples from children with meningitis.

CSF specimens from children suffering from meningitis, aged to 12 years, were included in the study if at least four of the following seven indicators of disease were in evidence: first, the presence of clinical features, such as gradual loss of playful activity, irritability, clouding of consciousness, convulsions, neck stiffness, and cranial nerve or motor defects; second, a CSF lymphocyte count at admission greater than 10 × 10⁶/liter; third, a CSF protein level at admission greater than 80 mg/dl; fourth, a CSF glucose/blood glucose ratio at admission of less than 0.5; fifth, contact with an intrafamilial adult positive for pulmonary TB; sixth, induration of 10 mm or more on tuberculin testing with 1 TU of purified protein derivative; and seventh, positive radiological features of primary complex in a chest radiograph in the form of hilar lymphadenopathy and/or consolidation with parenchymal infiltrate, segmental collapse, or miliary mottling.

The response to antituberculous treatment was taken as one of the clinical criteria in the final analysis, although CSF samples were collected before initiation of treatment.

CSF cell counting and smear examination of a centrifuged CSF sample were carried out after conventional Ziehl-Neelsen staining. To rule out CSF pyogenic meningitis, we performed Gram staining and culture by inoculation on chocolate and blood agars incubated at 37°C. CSF was centrifuged in a sterile container, and the pellet was resuspended in selective Kirchner liquid medium and processed for culture of mycobacteria in multiple media using standard procedures (7). Mycobacterial DNAs from the samples were amplified using the following primers: IS₆₁₁₀ a (5'-CCTGCGAGCGTAGGGCGG-3') and IS₆₁₁₀ b (5'-CTCGCTCCAGGCGCGTTCCGG-3') and TRC₄ primer 1 (5'-GACAACGAGCTGCGCTACT-3') TRC₄ primer 2 (5'-GACGGAATTAGCGTAGCTCC-3').

The IS₆₁₁₀ primers amplify a fragment with a length of 123 bp, while the 18-mer TRC₄ primers amplify a fragment with a length of 173 bp. This fragment is located in open reading frame Rv0697 in the genome of M. tuberculosis published by Cole et al. (3) and is a single target of the repetitive element TRC₄.

We examined concordance between PCR results and clinical signs of TB meningitis, as well as between the two PCRs, using bivariate two-by-two tables. The level of agreement between the methods was assessed by kappa statistics and disagreement using McNemar’s test. Results were considered statistically significant when the P value was less than 0.05.

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Six patients whose samples were found to be positive by PCR using TRC4 primers were diagnosed as having TB meningitis between the results of PCR using IS6110. The crude agreement was 80%, and the chance-corrected agreement was 55%. There was moderate agreement between the two methods (kappa = 0.53). However, PCR with TRC4 primers had a higher sensitivity than PCR with IS6110 primers in detecting clinically positive cases.

Of the 20 confirmed TB meningitis cases, PCR using IS6110 primers failed to detect one case while PCR using TRC4 primers was positive in all 20 cases. Of the eight samples from patients with confirmed non-TB meningitis, six were negative by PCR and two were repeatedly positive by PCR using both probes.

While PCR has been previously used to diagnose TB meningitis (6, 9, 12, 13), most researchers have reported using insertion element IS6110 primers. We used a new target for PCR in an area where 40% of the M. tuberculosis organisms carry a single copy of IS6110 and 4% do not carry even a single copy of IS6110 (4). We found that the sensitivity of PCR can be increased by using two probes (IS6110 and TRC4). In our study, there were fewer false-negative results by PCR with TRC4 (9%) than by PCR with IS6110 (22%). The higher false negativity of PCR with IS6110 may in part be due to the absence of IS6110 copies.

The strength of our study is that all 20 of the culture-positive samples were also positive by PCR using TRC4 primers. PCR was positive for one child with undisputed evidence on autopsy. PCR was also positive using both probes for two patients who had lymph node biopsies and skin biopsies positive for TB. Among the eight non-TB meningitis patients who were proven not to have TB by non-acid-fast-bacillus cultures, six were negative by both probes. The remaining two were positive by both probes. In these two cases, the possibility of coinfection with M. tuberculosis cannot be ruled out.

The two samples from patients who were clinically positive and responded to antituberculous therapy were negative by PCR using both probes. The negative PCR results in these cases may have been due to paucibacillary CSF from TB meningitis or due to the effect of an unknown history of prior antituberculous therapy.

The main limitation of our study is that currently there is no diagnostic test to serve as an adequate “gold standard” to evaluate PCR. While culture has low sensitivity, clinical assessment may be subjective. The test still has problems with sensitivity and specificity. The increased sensitivity may come at the expense of decreased specificity.

We found that using one more sets of primers especially from a repetitive element like TRC4 could be advantageous in increasing the sensitivity of PCR. Clinical parameters such as CSF sugar and protein levels, the CSF/blood glucose ratio, a primary tuberculous lesion in the lung, hilar lymphadenopathy, miliary paucification, and calcification are not sufficient in detecting TB meningitis. Cranial imaging computerized axial tomography and magnetic resonance imaging have the potential to study the pathological changes occurring in TB meningitis but are prohibitively expensive for developing countries and continue to be surrounded by uncertainty. Further research is needed to simplify PCR and to convert it into a cost-effective technique that can be included even in an unsophisticated laboratory of a developing country.
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