Simultaneous Use of Two PCR Systems Targeting IS6110 and MPB64 for Confirmation of Diagnosis of Tuberculous Lymphadenitis


Abstract

PCR has emerged as a powerful technique for detection of various pathogens including *Mycobacterium tuberculosis*. In present study, eighty one samples of lymph node biopsies from clinically suspected cases of tuberculous lymphadenitis were examined for AFB, culture on Löwenstein Jensen medium and simultaneous use of two PCRs targeting IS6110 and MPB64. Positivity with *M. tuberculosis* culture and AFB was 13.6% and 28.4% respectively. All samples culture positive for nontuberculous mycobacteria were negative by both PCR systems. Higher proportion of positive results were observed with PCR targeting IS6110 by which 56 of 81 (69.1%) samples showed positive results as compared to PCR targeting MPB64 by which 39 of 81 (48.2%) samples showed positive results. When combined, 63 out of 81 (77.8%) samples were detected positive for *M. tuberculosis* DNA. However, 7/81 (8.6%) samples remained negative by IS6110 but positive by MPB64 method. Thus our data suggest that the use of one additional PCR (other than IS6110 system) can reduce false negativity of PCR results in the samples harboring zero copy of IS6110 element which is known to exist in Indian population.

Key words: IS6110, MPB64, Gene amplification, Tuberculous lymphadenitis
INTRODUCTION

The diagnosis of tuberculous lymphadenitis is confirmed by the presence of acid fast bacilli (AFB), presence of characteristic granuloma and by isolation of Mycobacterium tuberculosis on culture. AFB cultures have been reported positive in 28% to 65% of patients in published studies. More over the culture takes six to eight weeks and this causes delay in the start of treatment. PCR based assays can help in early diagnosis of tuberculosis. There is a definite need for improving the sensitivity of techniques for the diagnosis of tuberculous lymphadenitis.

Several reports have demonstrated the value of PCR in the diagnosis of mycobacterial lymphadenitis. Most commonly used method targeting insertion sequence IS6110 has danger of missing at least 10% of Indian tuberculosis patients harboring zero copy number of IS6110. Therefore there is need to know the comparative value of other PCR systems when simultaneously used for improving diagnosis.

In this study we have used two PCR systems targeting IS6110 and MPB64, which have earlier been found to be useful in the early diagnosis of tuberculosis.

MATERIAL AND METHODS

Eighty one samples of lymph node biopsies from patients clinically diagnosed as having tuberculosis lymphadenitis, collected during September 2002 to April 2003, were included in the study. The clinical symptoms suggestive of tubercular lymphadenitis were fever, anorexia or weight loss, and lymphadenopathy. Biopsies were collected from AIIMS, New Delhi, in clean and sterile containers and transported in cooled condition to National JALMA Institute for Leprosy and Other Mycobacterial Diseases (ICMR), Tajganj, Agra. Biopsies were grinded in 1 ml of 0.85% saline buffer and cultured on Lowenstein Jensen medium. DNA was isolated by a physicochemical method using lysozyme and proteinase K being routinely used at our laboratory.

Two PCR systems for amplification of M. tuberculosis sequence were used

PCR 1 (System targeting IS 6110) - DNA amplification was performed using IS1 (5’ CCT GCG AGC GTA GGC GTC GG 3’) and IS2 (5’ CTC GTC CAG CGC CGC TTC GG 3’) that specifically amplified 123 bp fragment of IS6110. Each PCR reaction contained 1X buffer, 200 µM dNTPs, 1.5 U Taq Polymerase, 200 ng of each Primers and 5 µl of sample DNA per 50 µl of reaction volume. DNA amplification was performed for 35 cycles following an initial denaturation at 94°C for 5 min. in a thermal cycler (GeneAmp PCR system 9700) by using following programme: denaturation at 94°C for 1.5 min, annealing at 70°C and extension at 72°C for 1.5 min with a final extension of 10 min at 72°C. The amplified product was stored at 4°C till the detection by electrophoresis.

PCR 2 (System targeting MBP 64)-DNA amplification of 240 bp fragment of MPB64 gene was performed using primers P1 (5’ TCC GCT GCC AGT CGT
nontuberculous mycobacteria were negative by both PCR systems (Table 1). By applying the two PCR assays to these samples from patients with clinical suspicion of tuberculosis lymphadenitis we found various degrees of reactivity in each of the PCRs. The overall higher proportion of positive results were detected with PCR assay 1 (targeting IS6110) by which 56 of 81 (69.1%) showed positive results as compared to PCR assay 2 (targeting MPB64) by which 39 of 81 (48.2.2%) samples showed positive results (table 2). When combined in 63 out of 81 (77.8%) samples, at least one of PCRs made it possible for us to detect M. tuberculosis DNA in samples (Table 1 & 2).

DISCUSSION
Extra pulmonary tuberculosis is often difficult to diagnose because of its diverse clinical presentations. In a significant proportion of clinical samples, low isolation rate due to low limit detection was observed by conventional techniques such as acid-fast staining and bacterial culture.

Aside from the improvements to and the increasing use of PCR for the diagnosis of the tuberculosis, application data on comparison of commonly used PCR assays in India is needed. In the present study two such methods targeting IS 6110 and MPB64 have been compared in diagnosis of tuberculous lymphadenitis. The results of the present study show that the system targeting insertion sequence IS6110 appears to be a better target for amplification of DNA from lymph node samples as compared to MPB64.
are thus less prone to fragmentation. In the present study, we used two PCR techniques to identify the causative agent in tuberculous lymphadenitis. When results of both the systems were combined positive signals were obtained in lymph node biopsies from 63 (77.8%) of 81 patients with clinical and histopathological diagnosis of tuberculous lymphadenitis. The positive signals obtained in these biopsies were higher with IS6110 system 56/81 (69.1%) than with MPB64 system 39/81 (48.2%). Beside 7/81 (8.6 %) samples were negative by IS6110 but were positive by MPB64 method. This could be due to absence of IS6110 copies, which is known to exist in Indian M. tuberculosis strains.

<table>
<thead>
<tr>
<th>Method</th>
<th>Number of patients</th>
<th>% Positive (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>81</td>
<td>21.0 (17)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(11 M. tb + 6 NTM)</td>
</tr>
<tr>
<td>AFB smear</td>
<td>81</td>
<td>28.4 (23)</td>
</tr>
<tr>
<td>PCR (IS 6110+MPB 64)</td>
<td>81</td>
<td>77.8 (63)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of samples</th>
<th>PCR 1 (IS 6110)</th>
<th>PCR 2 (MPB 64)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>+</td>
<td>+</td>
<td>39.5%</td>
</tr>
<tr>
<td>24</td>
<td>+</td>
<td>–</td>
<td>29.6%</td>
</tr>
<tr>
<td>7</td>
<td>–</td>
<td>+</td>
<td>8.6%</td>
</tr>
<tr>
<td>18</td>
<td>–</td>
<td>–</td>
<td>22.2%</td>
</tr>
<tr>
<td>Total positives</td>
<td>56 (69.1%)</td>
<td>39 (48.2%)</td>
<td></td>
</tr>
<tr>
<td>With single PCR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total positive with both PCR</td>
<td></td>
<td>63 (77.8%)</td>
<td></td>
</tr>
</tbody>
</table>
Thus our data strongly support the use of one additional PCR other than IS6110 system so as to reduce false negativity in the samples harboring zero copy of IS6110 element which is known to exist in Indian *M. tuberculosis* strains. PCR positivity results on such samples have been reported between 83%-87.5% using IS6110 system\(^5,15,16\). However, 55% positivity in similar specimens has been reported using devR PCR system\(^4\). Based on these experiences we recommend the use of more than one system in diagnosis of tuberculosis one of which could be IS6110 system.

**ACKNOELGDEMENT**

Authors are thankful to Dr. M. Singh of AIIMS for the clinical specimens and to LEPRA, UK for partial financial support to undertake this study. Pushpendra Singh and GPS Jadaun are research scholars with fellowships from CSIR New Delhi.

**REFERENCES**


