PCR detection of co-infection with *Mycobacterium tuberculosis* and *Mycobacterium avium* in AIDS patients with meningitis

Members of the *Mycobacterium tuberculosis* complex (MTC) are an important cause of morbidity and mortality in India (Barani *et al.*, 2012; Gupta *et al.*, 2011). However, in recent years, the significance of nontuberculous mycobacteria (NTM) has also increased after the onset of the HIV/AIDS epidemic (Karne *et al.*, 2012; Narang, 2008). Meningitis due to *Mycobacterium avium* is rare but often fatal, resulting in death in as many as 60% of cases. Literature regarding the true prevalence of dual infection with *M. tuberculosis* and *M. avium* is limited and most of these cases are treated as drug-resistant cases of tuberculosis (TB). Here, we report two cases of dual infection with *M. tuberculosis* and *M. avium*, both of which had a fatal outcome. This correspondence highlights an urgent need for the use of multiplex PCR in routine diagnostic laboratories. This would help in the diagnosis of co-infections due to NTM, especially *M. avium* and *M. tuberculosis*, in patients with HIV/AIDS and may, therefore, assist in better patient management and outcome of the disease.

The first case was a 58-year-old male patient who presented with a history of fever along with cough and expectoration during the previous month and a half. The fever was intermittent and low grade with a rise of temperature in the evening. There was a history of altered sensorium for the past 18 days and abdominal distension for the past 5–6 days associated with constipation and decreased urine output. The patient also had a history of weight loss for 3 months. The patient had a known case of cortical venous thrombosis, which occurred 2 years previously, for which he was on oral anticoagulants. He was a smoker, and had been for the past 25 years, consuming two bundles of ‘beedi’ (locally made cigarettes made from tobacco leaves) per day. There was also a history of alcohol consumption over the last 25 years. On examination, the pulse rate was 76 min⁻¹, blood pressure was 140/90 mm Hg and respiratory rate was 18 min⁻¹. The patient was disoriented to time, place and person. There was neck rigidity and the Kernig’s and Brudzinski’s signs were positive. Local examination revealed spider naevi on the posterior chest wall, palmar erythema, gynaecomastia and parotidomegaly, indicating liver failure. On per-abdomen examination there was hepatomegaly 3 cm below the right costal margin. During the present admission the patient was diagnosed as HIV-positive.

The details of the laboratory investigation of his blood and cerebrospinal fluid (CSF) are given in Table 1. The patient was started on anti-tubercular treatment (ATT) but succumbed to his illness on the second day of admission.

The second case was a 38-year-old HIV-infected male who had been receiving antiretroviral therapy for the previous 2 years and presented to the emergency outpatient department of our institute with a history of very severe headache for the past 6 days, which was continuous and more severe at night. There was no postural variation of the headache. There was no history of cough or weight loss. He was treated for pulmonary tuberculosis 6 months previously and had completed his course 2 weeks before presenting to the hospital. On examination, the pulse rate was 60 min⁻¹, blood pressure was 140/80 mm Hg and respiratory rate was 20 min⁻¹. Systemic examination was within normal limits except that he had neck rigidity. The details of the laboratory investigation of his blood and CSF are given in Table 1. The patient succumbed to his illness on the day of admission, before any treatment could be initiated.

Culture and identification of the mycobacteria isolated from the CSF were performed according to standard protocols (Watt *et al.*, 1996). In both cases direct microscopy with ZN stain was negative. Case 1 was positive for culture of both *M. tuberculosis* and *M. avium*, whereas in case 2, culture was negative for both organisms. In addition, India ink wet mount for capsulated yeast was positive in case 2, along with a positive latex agglutination test for cryptococcal antigen.

DNA was extracted from 200 μl CSF from each of the patients by using a commercially available DNA extraction kit (QIAamp DNA minikit, Qiagen), as per the protocol provided by the manufacturer. The eluted DNA was stored at −20 °C until use. Multiplex PCR was performed by using IS6110 primers specific for MTC and IS1245 primers specific for *M. avium* as described previously (Brisson-Noël *et al.*, 1989; Hatta *et al.*, 2010). IS6110 and IS1245 are two of the most commonly used PCR targets for the detection of MTC and *M. avium*, respectively, as these are robust, sensitive and specific; however, strains which do not carry these genes do occur in some parts of the world, including India (Chauhan *et al.*, 2007), therefore, the inclusion of additional targets might be advisable (Kusum *et al.*, 2011). The PCR mixture contained the following: 10 × PCR buffer, 10 mM dNTPs, 10 μM each IS6110 primers (forward and reverse), 10 μM each IS1245 primers (forward and reverse), 5 U μl⁻¹ Taq polymerase, DNA template ( neat and in 1:10 dilution, as is routine practice at our laboratory so as to dilute out inhibitors from patient samples) and water. DNA amplification was performed by using the following program: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min and extension at 72 °C for 1 min, with a final extension step at 72 °C for 10 min. The amplified products were run on a 1.5% agarose gel and examined under UV light. DNA of *M. tuberculosis* strain H37Rv and an MTCC strain of *M. avium* were used as positive controls and PCR...
grade water was used as a negative control. Neither of the two samples showed PCR inhibition and the spike test was positive. The expected band size using the primers specific for \textit{M. tuberculosis} was 123 bp and the expected band size using primers specific for \textit{M. avium} was 187 bp. The DNA extracted from CSF samples of both the patients was positive for both \textit{M. tuberculosis} and \textit{M. avium}. The IS\textsubscript{1245} insertion sequence element may rarely be present in other members of the \textit{Mycobacterium avium} complex. Therefore, to confirm the identification of \textit{M. avium}, we sequenced the amplified product of the DNA extracted from the CSF of case 1. Nucleotide sequencing was performed with the Big Dye Terminator Cycle Sequencing kit, version 3.1 (Applied Biosystems), for both strands. All the sequencing reactions were purified and analysed on an ABI 3130 Genetic Analyzer (Applied Biosystems). The sequence obtained was compared with sequences available in the GenBank DNA database. The sequence of our isolate showed a 99\% sequence similarity to the ex-type strain of \textit{Mycobacterium avium} (accession no. CP000479.1). Sequence data for this isolate were submitted to the GenBank under accession no. JQ081272.1.

Meningitis due to \textit{M. avium} is rare and often fatal (Flor et al., 1996; Weiss et al., 1995). Co-infections due to \textit{M. tuberculosis} and \textit{M. avium} have rarely been reported in literature. There have been reports of pulmonary tuberculosis with superadded \textit{M. avium} infection in immunocompetent as well as immunocompromised patients (Khan et al., 2010). Co-infection with \textit{Mycobacterium avium} in case 2 could have contributed to the rapid progression of this acute episode and led to the fatal outcome.

This report adds a new dimension to the diagnosis of suspected tubercular meningitis in immunosuppressed individuals, which is a diagnostic dilemma, especially with the limitations of conventional diagnostic methods. If untreated, such co-infections are fatal. In spite of being on ATT, the patient in case 1 died, while the patient in case 2 died even before a diagnosis could be made, since the meningitis was considered to be only tubercular and no timely modification of treatment could be carried out.

Unresponsiveness to the ATT therapy under such circumstances is commonly taken as a clinical case of drug-resistant tubercular meningitis and prompts a shift in therapy from first-line to second-line

<table>
<thead>
<tr>
<th>Sample</th>
<th>Investigation</th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Haemoglobin (g ml(^{-1}))</td>
<td>13</td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td>Total leukocyte count (mm(^{-3}))</td>
<td>2800</td>
<td>6500</td>
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<tr>
<td></td>
<td>Differential leukocyte count (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neutrophil</td>
<td>80</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>Basophil</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Eosinophil</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Lymphocyte</td>
<td>18</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Monocyte</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Random blood sugar (mg dl(^{-1}))</td>
<td>127</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>CD4 count (mm(^{-3}))</td>
<td>ND</td>
<td>59</td>
</tr>
<tr>
<td>CSF</td>
<td>Total leukocyte count (mm(^{-3}))</td>
<td>20</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>Differential leukocyte count (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neutrophil</td>
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<td>30</td>
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<td>Basophil</td>
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<td>0</td>
</tr>
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<td>Eosinophil</td>
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</tr>
<tr>
<td></td>
<td>Lymphocyte</td>
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<td>70</td>
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<td></td>
<td>Monocyte</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Protein (mg dl(^{-1}))</td>
<td>53</td>
<td>600</td>
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<td>Sugar (mg dl(^{-1}))</td>
<td>32</td>
<td>63</td>
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<tr>
<td></td>
<td>Adenosine deaminase (U l(^{-1}))</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Culture of \textit{M. tuberculosis}/\textit{M. avium}</td>
<td>+/+</td>
<td>–/–</td>
</tr>
<tr>
<td></td>
<td>Multiplex PCR for MTC(IS\textsubscript{6110}) &amp; \textit{M. avium} (IS\textsubscript{1245})</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Cryptococcal antigen detection with latex agglutination</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>
drugs. However, overuse of second-line drugs has the known disadvantage of increasing drug resistance. In addition, in resource-poor developing countries, like India, this comes with an additional financial burden on the patient. Hence, in such cases of unresponsiveness to therapy, especially in a setting of HIV with low CD4 counts, an alternative etiology should be sought for and use of molecular methods like multiplex PCR should be encouraged as a routine procedure, rather than an exceptional one.

In conclusion, this correspondence highlights an urgent need for use of multiplex PCR in routine diagnostic laboratories. This would help in the diagnosis of co-infections due to nontuberculous mycobacteria, especially *M. avium* with *M. tuberculosis*, in patients with HIV/AIDS and would, therefore, assist in better patient management and outcome of the disease.

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