Multiplex PCR in diagnosis of *M. tuberculosis* and *M. avium* co-infection from lymph node in an AIDS patient

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Abstract

A 35-year-old, HIV-seropositive male (CD4 count 41 cells/mm^3) on highly active antiretroviral (HAART) presented with fever and weight loss for 3 months and new skin lesions. He was earlier diagnosed of TB and was on anti-tubercular therapy (ATT). The retroperitoneal lymph node aspirate showed acid-fast bacilli and epithelioid cell granulomas; however, cultures remained sterile. A dual infection with *Mycobacterium tuberculosis* and *Mycobacterium avium* was diagnosed with multiplex polymerase chain reaction (MPCR). Clarithromycin was added to ATT, and on follow-up at 1 and 3 months, the patient responded well. Molecular methods like MPCR should be exploited for routine diagnosis of high-risk patients.

Key words: *Co-infection, multiplex polymerase chain reaction, Mycobacterium avium, Mycobacterium tuberculosis*

Introduction

Mycobacterium tuberculosis is an important cause of morbidity and mortality worldwide. In recent years, the significance of non-tuberculous mycobacteria (NTM) has increased due to the AIDS pandemic.[1-3] Among the NTM, *Mycobacterium avium-intracellulare* complex (MAC) is the most common group, associated with more than 60% mortality in AIDS patients. For diagnosis, conventional microscopy and culture play a limited role because of prolonged incubation period and low sensitivity. At a majority of centres in developing countries, mycobacterial isolates are not identified to species level and patients are treated as TB cases or multidrug-resistant (MDR) TB cases. Nucleic acid amplification tests (NAAT) can play an important role in rapid specific diagnosis and timely initiation of treatment for better patient outcomes. The diagnostic potential of NAAT like multiplex-PCR (MPCR) has rarely been utilised for routine diagnosis. Earlier we have reported the use of MPCR for rapid diagnosis of *Mycobacterium tuberculosis* and *Mycobacterium avium*.[4] Here, we present a case of dual infection with *M. tuberculosis* and *M. avium* from retroperitoneal lymph node in an AIDS patient with low CD4 counts.

Case Report

A 35-year-old, HIV-seropositive male on highly active antiretroviral (HAART) presented to our tertiary care hospital with history of fever and weight loss for last 3 months and new skin lesions. He had been diagnosed of TB earlier and was on anti-tubercular therapy (ATT). In the present admission, his CD4 count was 41 cells/mm^3. A computed tomography (CT) scan revealed multiple enlarged retroperitoneal lymph nodes with thickening and oedema of walls of jejunum, ileum and transverse colon. Ultrasound-guided fine needle aspiration (FNA) was done from the nodes. The aspirate was particulate; air-dried smears were prepared and stained with May-Grünwald Giemsa (MGG) stain. Part of the sample was subjected to microbiological diagnosis by Ziehl–Neelsen's (ZN) acid-fast staining, Löwenstein–Jensen medium (LJ) and BACTEC MGIT 960 culture, and home-brew MPCR which targets *M. tuberculosis* and *M. avium*.[4]

The smears were cellular and showed sheets of foamy macrophages and polymorphs. Negative shadows of bacilli within the macrophages as well as in the background, with occasional epithelioid cell granulomas could be appreciated in MGG-stained smears, and ZN stain was strongly positive for acid fast bacilli (AFB) [Figure 1]. However, the culture remained sterile after 6 weeks. MPCR was carried out in duplicate using IS*6110* primers specific for *M. tuberculosis* complex and IS*1245* primers specific for *M. avium*, as described previously.[4] The sensitivity of MPCR for diagnosis of *M. tuberculosis* and *M. avium* was 10 fg, i.e. equivalent to 1–2 bacilli. The specimen showed specific bands for both *M. tuberculosis* (123 bp) and *M. avium* (187 bp) [Figure 2]. Standard strains of *M. tuberculosis* H37Rv and an MTCC strain of *M. avium* were used as positive controls, and PCR-grade water as negative control. The patient was immediately started on anti-MAC regimen with clarithromycin, along with the ongoing ATT. On follow-up at 1 and 3 months, a favourable clinical response was observed and the lesions considerably reduced in size and severity.

Discussion

Although *M. tuberculosis* and MAC are common opportunistic infections in patients with AIDS, there

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Received: 27-11-2013
Accepted: 26-07-2014

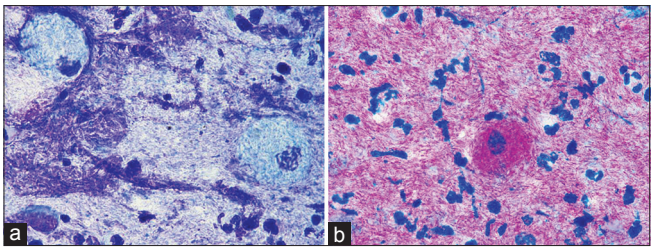

Figure 1: (a) Micro-photograph showing many negative shadows of bacilli (May-Grünwald Giemsa stain, ×100); (b) Many acid-fast bacilli within the macrophage as well as extracellularly (Ziehl–Neelsen's stain, ×100)

have been rare reports of dual infection. To the best of our knowledge, this is the first case report of dual infection with *M. tuberculosis* and *M. avium* from retroperitoneal lymph node in an AIDS patient. Damian *et al.* reported co-infection of *M. tuberculosis* and MAC in an immunocompetent host from pulmonary specimen; however, the dilemma whether both were pathogens or colonisers in the host remained unresolved.[5] Another report described a case of cervical lymphadenopathy where initial cultures grew MAC resistant to the standard anti-tuberculosis agents. Later, the culture of excised lymph node grew *M. tuberculosis* and treatment was modified.[6] Both these cases depended on conventional methodologies like staining and culture, and hence the delay in diagnosis and treatment.

It is difficult to attribute significance when MAC is isolated in cultures since these are ubiquitous environmental microorganisms commonly found in respiratory specimens and as laboratory contaminants. Co-isolation of MAC along with *M. tuberculosis* from pulmonary specimens is, therefore, generally labelled as *M. tuberculosis* infection and treated accordingly. The American Thoracic Society (ATS) guidelines to diagnose pulmonary disease caused by NTM is based on the exclusion of *M. tuberculosis*;[7] however, in sterile specimens like lymph node aspirates, the presence of both *M. tuberculosis* and NTM denotes significance to both organisms. When only conventional diagnostic methods like microscopy and culture are used, many cases may be missed. There can be situations when microscopy is positive but culture is negative, and since acid fastness cannot differentiate between *M. tuberculosis* and NTM, it does not help in narrowing down the specific therapy to target *M. tuberculosis* or NTM, or to target both as in our case. A positive microscopy from sterile specimens warrants ATT, but when such cases fail to respond to ATT, as in the present case, an attempt to look for other causative pathogens can be made. If patients on ATT do not respond to therapy, they are generally started on MDR-TB treatment; however, this worsens the problem as the therapy of NTM is different from that of *M. tuberculosis*. Here, NAAT can play a vital role in rapid diagnosis as well as for differentiating *M. tuberculosis* from NTM, which may be beneficial in initiating the right drug. An advanced diagnostic approach which can help in such cases is MPCR, though it has not been deployed for routine diagnostic use at a majority of centres.

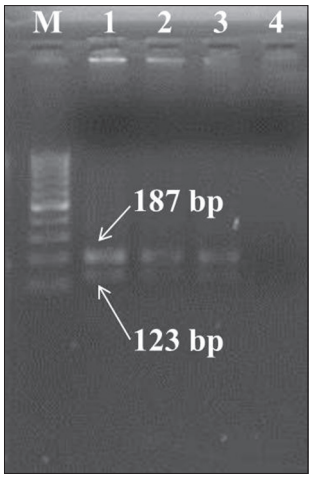

Figure 2: MPCR showing specific bands for both *M. tuberculosis* (123 bp) and *M. avium* (187 bp). Lane 1: Positive control; lanes 2 and 3: Sample in duplicate; lane 4: negative control; lane M: 100 bp DNA ladder

In conclusion, with increase in awareness of NTM as pathogens, the clinical index of suspicion should be high, especially in AIDS patients with low CD4 counts.[8] Moreover, when there is non-response to therapy with ATT, an alternative diagnosis should be sought for. Our patient had been treated only for *M. tuberculosis* earlier and was clinically worsening, but as soon as the diagnosis of dual infection was made, he was started on clarithromycin to which he responded well. A dual infection could only be pointed out with the use of MPCR and corrective intervention could be taken on time. Hence, we would like to emphasise that the diagnostic potential of molecular methods like MPCR should be exploited beyond research studies, for routine diagnosis of patients at high risk of infections.

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Quick Response Code:

Website: www.ijmm.org

DOI: 10.4103/0255-0857.150940

How to cite this article: Sharma K, Mewara A, Gupta N, Sharma A, Varma S. Multiplex PCR in diagnosis of *M. tuberculosis* and M. avium co-infection from lymph node in an AIDS patient. Indian J Med Microbiol 2015;33:S151-3.

Source of Support: Nil, **Conflict of Interest:** None declared.