Summary

Background: There is high prevalence of tuberculosis in patients with HIV infection; hence the role of non-tuberculous mycobacteria (NTM) in HIV patients has always been undermined. NTM may be responsible for clinical disease in a substantial number of immuno-compromised HIV sero-positive individuals even in a country endemic for Mycobacterium tuberculosis (M. tuberculosis). The study was designed to look for the contribution of NTM to morbidity in HIV seropositive patients.

Material and Methods: In a prospective study of ninety-four HIV seropositive individuals presenting with pulmonary or extra-pulmonary symptoms suggestive of mycobacterial infection, appropriate samples were collected and processed. Detailed clinical history was utilized to differentiate colonization or contamination by NTM from true lung disease.

Results: Fourteen samples grew mycobacterial species, 8(57.2%) being NTM. The distribution of NTM was- 3 M. avium complex, 2 M. fortuitum, 2 M. vaccae, 1 M. phlei. 6 isolates were M. tuberculosis.

Conclusion: NTM may be responsible for a significant proportion of mycobacterial infections in HIV seropositive individuals. Despite the high endemicity of tuberculosis in developing countries like India, the presence of NTM should be ruled out especially in immuno-compromised HIV seropositive individuals before instituting anti-tubercular therapy empirically. In addition, non-response of NTM to ATT may be wrongly attributed to multi-drug resistant tuberculosis.

Key words: Non-tuberculous mycobacteria, Immuno-compromised, HIV sero-positive individuals

INTRODUCTION

The pandemic of HIV infection has brought considerable change in the epidemiology of mycobacterial infections the world over. While it has led to the resurgence of M. tuberculosis in USA and Europe, it has also been associated with an increase in infections due to non-tuberculous mycobacteria (NTM).1.

The prolonged and profound immuno-suppression of cell mediated immunity that characterizes AIDS provides opportunity for relatively avirulent NTM to cause disease. Mycobacterium avium complex (MAC) is now the commonest cause of systemic bacterial infections in AIDS patients in the United States and other developed countries.1 In developing countries, especially India, where tuberculosis is endemic, M. tuberculosis has been reported to be the main secondary mycobacterial infection in AIDS patients. Little information exists about NTM disease incidence, mainly because of the resource poor health care system and the absence of mycobacterial culture.2 Studies to assess the clinical significance and disease spectrum of NTM in HIV seropositive individuals are far and few.

In this study, HIV seropositive patients were investigated for mycobacterial infections. All mycobacterial isolates were identified and their drug susceptibilities determined.

MATERIAL AND METHODS

The study was conducted over a period of 18 months. Ninety-four HIV seropositive patients (positive for HIV 1and / or 2 by 3 ELISA kits as per NACO guidelines), who presented with suggestive
clinical and radiological evidence of possible pulmonary or extra-pulmonary mycobacterial infection were included in the study after taking their informed consent¹. Appropriate clinical samples were collected depending upon signs and symptoms, hence the likely system involved. Early morning well coughed out sputum specimens, broncho alveolar lavage, bronchial wash and endotracheal aspirate specimens were received from patients with clinical and radiological findings suggestive of pulmonary tuberculosis. Entire early morning urine specimens were received from patients suspected of urinary tuberculosis. Sterile body fluids such as cerebrospinal fluid (CSF), blood, pleural fluid and others were collected from patients suspected of disseminated mycobacterial infections. Biopsy specimens were obtained from patients with lymphadenitis. One hundred and five specimens comprising 80 expectorated sputum specimens, seven BAL specimens, 11 cerebrospinal fluid (CSF) specimens, three blood specimens, two each of urine and pleural fluid, one pericardial fluid specimen and one gastric biopsy specimen were collected. All specimens were collected with aseptic precautions in sterile leak proof containers and transported to the laboratory.

The specimens from 'unsterile' sites, such as sputum, urine and gastric juice were decontaminated by the Petroff’s method². Sterile fluid samples such as CSF were concentrated by centrifugation. The blood samples were processed by lysis centrifugation method using 0.05% saponin solution³.

About 250µl of sediment was inoculated onto a pair of Lowenstein Jensen slants (LJ) containing glycerol and pyruvate. The inoculated media were incubated at 37°C and observed periodically for growth. In addition, 500µl of the sediment was also inoculated into BACTEC 12 B medium (Becton- Dickinson diagnostic instrument systems, Maryland, U.S.A.).

All mycobacterial isolates were identified to species level according to growth rate, growth at temperatures of 20, 37 and 42 °C, colony morphology, pigmentation, photoreactivity and a battery of biochemical tests⁴ (niacin production, nitrate reduction, semiquantitative catalase, Tween 80 hydrolysis, tellurite reduction, arylsulfatase and urease production, NaCl tolerance, growth on MacConkey agar). Drug susceptibility profiles of all mycobacterial isolates were determined. For the slow growers, susceptibility to isoniazid (INH), rifampicin, ethambutol and ciprofloxacin was determined by proportion method⁵. The standard reference H₃RV strain was included in each batch. For rapid growers (M. fortuitum, M. vaccae, and M. chelonae), MICs were determined by microbroth dilution method against amikacin, erythromycin, azithromycin, ciprofloxacin and doxycycline⁶.

**RESULTS**

Maximum patients (82%) were in third and fourth decades of their life with male preponderance (91%). Heterosexual contact (74%) especially with commercial sex workers was the common source of infection followed by blood and its product

| Table 1: Characteristics of 14 patients with M. tuberculosis and NTM isolates |
|---------------------------------|--------|--------|
| Clinical or radiological features| M. tb | NTM |
| Prolonged fever (> 1 month duration) | 6 | 8 |
| Cough | 5 | 8 |
| Weight loss | 6 | 8 |
| Haemoptysis | None | None |
| Radiographic cavitating disease | None | None |
| Alveolar infiltrates | 4 | 6 |
| Adenopathy | 1 | None |
| Effusions | 1 | None |
| Normal chest radiology* | 1 | 2 |
| Microscopy positive for AFB | 5 | 6 |
| CD4 Counts (< 200/mm³) | 5** | 7 |

* One patient with M. tuberculosis CD4 counts: 142/mm³. 
One patient with MAC isolate in the NTM group 
** In one patient with M. tuberculosis, CD4 counts could not be done.
transfusion (16%) and intravenous drug usage (9%) for HIV seropositivity.

Prolonged fever (>1 month duration) and weight loss (≥ 10 kg body weight) were the most common presenting symptoms present in all patients with mycobacterial infections followed by cough with expectoration (Table I). None of the patients had haemoptysis.

25/94 (26.8%) of HIV seropositive patients were found to have mycobacterial infections. There were a total of 17 isolates, of which 14 were identified to species level (three isolates were lost due to media spoilage). Eight samples were smear positive but culture negative. Of these eight patients, four were on ATT and one on isoniazid prophylaxis. Six isolates (42.8%) were found to be M. tuberculosis and eight isolates (57.2%) were identified as NTM. The species distribution of NTM was: M. avium complex (MAC) (n= 3), M. fortuitum (n= 2), and M. vaccae (n= 2), M. phlei (n=1). Five of the 6 M. tuberculosis isolates were from sputum and one was isolated from pleural fluid while all NTM species were isolated from sputum. 6/8 NTM isolates (Table: 2) were detected on smear as well as isolated in culture.

On chest radiography, cavitating disease pattern was not seen in any group (M. tuberculosis and NTM), however, alveolar infiltrates were found in both groups (Table I).

Table 2: Clinico-Microbiological profile of eight patients with NTM isolates.

<table>
<thead>
<tr>
<th>No.</th>
<th>Age/Sex</th>
<th>Isolate species</th>
<th>No. of sputum samples submitted (n=6)</th>
<th>No. of occasions direct microscopy positive</th>
<th>No. of occasions culture positive</th>
<th>CD 4 counts/µl</th>
<th>Radiological features</th>
<th>Other features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>30/M</td>
<td>MAC</td>
<td>3</td>
<td>2</td>
<td>113</td>
<td>NAD</td>
<td>Oc +ve</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>35/M</td>
<td>M. phlei</td>
<td>3</td>
<td>1</td>
<td>455</td>
<td>NAD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>18/M</td>
<td>MAC</td>
<td>2</td>
<td>1</td>
<td>147</td>
<td>C +ve Generalized lymphadenopathy, hepatosplenomegaly</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>23/F</td>
<td>MAC</td>
<td>1</td>
<td>2</td>
<td>147</td>
<td></td>
<td>Patchy parenchymal opacities</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>40/M</td>
<td>M. vaccae</td>
<td>2</td>
<td>2</td>
<td>57</td>
<td></td>
<td>Bilateral lower zone interstitial infiltrates</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>37/M</td>
<td>M. fortuitum</td>
<td>3</td>
<td>2</td>
<td>151</td>
<td></td>
<td>Bilateral diffuse parenchymal opacities</td>
<td>Taken ATT twice in the past</td>
</tr>
<tr>
<td>7.</td>
<td>45/M</td>
<td>M. vaccae</td>
<td>2</td>
<td>2</td>
<td>81</td>
<td></td>
<td>Patchy parenchymal opacities</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>28/M</td>
<td>M. fortuitum</td>
<td>3</td>
<td>2</td>
<td>110</td>
<td></td>
<td>Bilateral Lower and middle zone opacities</td>
<td>C +ve Generalized lymphadenopathy</td>
</tr>
</tbody>
</table>
Three of five *M. tuberculosis* isolates were sensitive to all four drugs tested (INH, rifampicin, ethambutol and ciprofloxacin). Resistance was seen in two isolates. Of these two isolates, one was resistant to INH and rifampicin (Multidrug resistant) while the other was resistant to ethambutol and ciprofloxacin.

All the three MAC strains were resistant to INH. Two were also resistant to rifampicin and ethambutol as well. The third strain was resistant to ciprofloxacin.

All the five rapid growers were sensitive to amikacin only and variably resistant to other drugs such as doxycycline, erythromycin and azithromycin.

**DISCUSSION**

Worldwide, there is an increasing awareness of the role of NTM as pathogens causing pulmonary disease or disseminated disease. Among NTM, MAC has been reported most commonly, followed by rapid growers and *M. kansasii*.

The exact disease burden of NTM infections still remains unclear in India. These infections are under diagnosed in many laboratories due to lack of proper culture facilities. Previous studies from India have documented presence of NTM in different clinical specimens at a varying frequency. Chakrabarti et al from Chandigarh isolated NTM in 7.4% of clinical specimens and *M. fortuitum* was the commonest isolate. Paramasivam et al from Chennai, South India reported 8.6% of NTM from sputum specimens of patients in BCG trial area. *M. avium* / *intracellulare* was the species most frequently isolated in their study. Das et al reported isolation of 8.3% NTM from various clinical specimens from Delhi and Kasauli. In a recent study from Vellore, 3.9% of all mycobacterial isolates were identified to be NTM. Pu, biopsy specimens and sputum specimens yielded most of the NTM, of which *M. chelonae* (46%) and *M. fortuitum* (41%) accounted for the majority. However, in most of these reports, clinical significance of these bacteria couldn’t be determined as these bacteria were isolated from single specimens. Besides, the HIV seropositivity status of the patients with NTM isolates in these studies was not known. Recently, Narang et al utilized paraffin slide culture technique to isolate NTM from 80 stools and 42 sputum samples from HIV seropositive tuberculosis patients. MAC was isolated from stool in four cases and from sputum in two cases while *M. fortuitum* was isolated from stool in two cases. In another study from the same institute, of 67 blood samples from HIV patients with suspected pulmonary or extra pulmonary tuberculosis, MAC and *M. simiae* were isolated in three patients each, while NTM were isolated from none of HIV seronegative patients.

In the present study, HIV seropositive individuals were investigated for mycobacterial infections. All mycobacterial isolates were speciated and their antibiotic susceptibilities determined. Unlike most previous studies, clinical significance of the NTM isolates was ascertained by repeated isolation of these bacteria from patients. Besides, detailed clinical and radiological characteristics of the patients as well as their immunological parameters (CD4 counts) were also taken into account along with the microbiological parameters. Our study shows that NTMs are also important pathogens in immuno-suppressed HIV sero-positive patients, despite high endemicity of tuberculosis in India.

The isolation of NTM from a pulmonary source presents a diagnostic challenge as these patients may be infected with NTM without evidence of pulmonary disease. Such an infection may be transient but it may also reflect disseminated NTM disease or subclinical NTM pulmonary disease. In addition, some NTM species that are generally considered non-pathogenic have been associated with pulmonary disease in the HIV infected host. The American Thoracic Society (ATS) has published diagnostic criteria recommending repeated culture of specimens from non-sterile sites such as sputum before committing a patient to long term therapy with antibiotics. These guidelines recommend: (i) three positive sputum cultures with negative AFB smear results, (ii) at least two positive sputum cultures and one positive AFB smear in the presence of compatible clinical and radiological features.
Following the above ATS guidelines, isolation of *M. vaccae* in case number five (direct microscopy negative for acid fast bacteria on all three occasions and culture positive only on one occasion) and *M. phlei*, case number two (direct microscopy negative for acid fast bacteria and culture positive only on one occasion with a normal X-ray and CD4 counts of 455/mm³ are not suggestive of significant NTM pulmonary disease and suggest mere colonization rather than a pathogenic role.

Clinical and radiological parameters are not accurate in differentiating *M. tuberculosis* from NTM infection. Symptoms of NTM pulmonary infection are variable and non-specific such as chronic cough, sputum production and fatigue. Malaise, dyspnoea, fever, hemoptysis, and weight loss can also occur with advanced NTM disease³. These symptoms are not unlike those of tuberculosis. In this study, patients of both groups (NTM/ *M. tuberculosis*) couldn’t be distinguished on the basis of clinical symptomatology (Table 1). Similarly, radiological parameters weren’t useful either (Table 1). NTM usually tend to cause thin walled cavities with less surrounding parenchymal infiltrates. However, in compromised AIDS patients, radiographic findings vary and may include diffuse or focal infiltrates, cavitary lesions, nodular and hilar lymphadenopathy. Commonly, chest X-ray may be normal despite the presence of disseminated disease⁴. In advanced HIV infection, TB often also has an atypical presentation. Chest radiographs rather than having apical cavitary disease may reveal adenopathy, apical infiltrates and miliary disease and occasionally, sputum cultures are positive for *M. tuberculosis* despite the presence of a normal chest X-ray (This pattern was seen in one patient in this study, Table: I). Most patients with mycobacterial isolates (both *M. tuberculosis* and NTM) in this study had advanced HIV infection (CD4 counts < 200/mm³) Tables: 1 and 2. In a recent study from Delhi, infiltrative lesions on Chest X-ray were seen in 61.9% of tuberculosis patients with HIV¹⁴.

Microscopic examination of acid fast bacilli is not reliable in discriminating *M. tuberculosis* from NTM¹². Hence presumptive diagnosis of tuberculosis based on above parameters (clinical, radiological, and microscopic) in the absence of culture and speciation can be misleading resulting in institution of unnecessary and inappropriate treatment with ATT. Pattern of resistance and outcome of treatment of NTM infection are significantly different from those of tuberculosis ². In this study, three patients (one MAC and one *M. fortuitum*) were actually being treated for resistant tuberculosis. In a study from eastern India, NTM could be isolated in 17.4% of cultures from consecutive samples of patients with presumed fibrovascular pulmonary tuberculosis. In this study, 14/15 patients with NTM isolates were already being treated for tuberculosis¹⁵. It is well documented that patients with disseminated MAC and *M. genavense*, who are treated, survive longer than those who are not treated. So, early treatment of pulmonary NTM is important to prevent dissemination and also improve survival ¹⁶.

To summarize, the present study shows that even in developing countries, with endemic tuberculosis, non-tuberculous mycobacterial infection may not be as uncommon as earlier thought to be. A high index of suspicion of NTM pulmonary disease, particularly in immunocompromised AIDS patients corroborated with clinical, radiological and laboratory guided mycobacterial identification from repeated sampling, will help in institution of appropriate treatment and management.

REFERENCES


**INAUGURATION OF 58TH TB SEAL CAMPAIGN – ANDHRA PRADESH**

Under the auspices of the Tuberculosis Association of Andhra Pradesh, the 58th TB Seal Campaign was inaugurated on 2nd October, 2007 by His Excellency, the Governor of Andhra Pradesh, Shri N.D. Tiwari, in Raj Bhavan, Hyderabad. Dr. Ramesh Chandra, Director of Health, presided. Dr. T.V. Venkateswarulu, Honorary General Secretary of the Association, presented a report on the activities of the State Association. The Governor distributed the Rolling Shields, Institutional Awards, Special Awards and Mementoes. About 200 persons, including Medical Officers, para-medical personnel and invitees, attended the function.