

DRUG SUSCEPTIBILITY OF *MYCOBACTERIUM TUBERCULOSIS* IN PATIENTS WITH AIDS AT A TERTIARY CARE HOSPITAL IN NORTHERN INDIA

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Abstract. The study was done to determine the anti-tuberculosis drug resistance patterns of *Mycobacterium tuberculosis* (MTB) in AIDS patients. Fifty antiretroviral drug naïve new AIDS patients with clinical evidence of pulmonary tuberculosis and no previous history of tuberculosis were recruited. Baseline CD4 counts and plasma viral loads (PVL) were measured by flow cytometry and RT-PCR, respectively. Sputum samples were obtained from each patient and subjected to Ziehl-Neelsen staining and cultured on Lowenstein-Jensen medium and using the BACTEC 460 system (B460). Antimicrobial susceptibilities were tested in all isolates using the B460 system. The occurrence of MTB was found to be more common with a PVL $>4 \log_{10}$ copies/ml (odds ratio: 4.6). Of 15 MTB isolates, 8 (53.3%) had single drug resistance, 4 (26.7%) had multidrug resistance (MDR) and 1 (6.7%) had resistance to three drugs (non-MDR). Two isolates (13.3%) were sensitive to all the four drugs. Resistance to first line anti-tuberculosis drugs was found to be higher among AIDS patients with MBT.

Keywords: *Mycobacterium tuberculosis*, BACTEC 460 TB system, MDR-TB, AIDS

INTRODUCTION

Southeast Asia accounts for nearly 40% of all tuberculosis (TB) cases globally and 18% of HIV infected people live in this region (Sharma and Mohan, 2004). HIV infected patients with CD4 counts less than 200 cells/ μ l are at high risk for developing opportunistic infections (OIs), such as TB. Patients with low CD4 cell counts progress to AIDS at varying speeds

and develop different OIs indicating other risk factors affect the occurrence of OIs (Brambilla *et al*, 2001). One risk factor for acquiring OIs, including TB, is the viral load irrespective of the CD4 cell count (Williams *et al*, 1999).

A consequence of the HIV and TB co-epidemic is the increasing occurrence of drug-resistant TB. The accelerating and amplifying influence of HIV infection and the delay in recognition and diagnosis of tuberculosis contribute to outbreaks of MDR-TB among HIV infected patients (Prasad *et al*, 2005). This study was undertaken to determine the anti-tuberculosis drug resistance patterns among *Mycobacterium tuberculosis* (MTB) in AIDS patients.

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MATERIALS AND METHODS

This study was carried out among newly diagnosed HIV patients at the Department of Microbiology, Maulana Azad Medical College, New Delhi, India. Of the 407 new HIV cases diagnosed during the study period (February to November 2008), 156 (38.3%) were diagnosed as having AIDS with CD4 cell counts <200 cells/ μ l. Of these, 50 had clinical evidence of pulmonary tuberculosis with suggestive chest X-ray findings, but no previous history of TB. These were recruited into this study after giving informed consent.

On recruitment, blood samples were taken from each patient to assess the baseline CD4 count using flow cytometry with the FACSCount™ system (Becton Dickinson, San Josse, CA). Plasma virus loads (PVL) of HIV virus were measured by reverse transcription polymerase chain reaction (RT-PCR) using an AMPLICOR HIV-1 Monitor test, version 1.5 (Roche Diagnostics, Indianapolis, IN) following the manufacturer's instructions (range of 400-750,000 copies/ml).

Three sputum samples were obtained from each subject and examined with Ziehl - Neelsen (ZN) staining and cultured on L-J medium and in a BACTEC 12B vial. Isolates grown in the culture medium were identified for typical morphology using L-J medium and ZN staining. Positive growth (GI) in BACTEC 12B medium was confirmed with ZN staining. Mycobacterial isolates were evaluated with niacin and NAP (p-nitro- α -acetyl-amino- β -hydroxy-propiofenone) tests for identification. Antimicrobial susceptibility testing (AST) of all isolates was performed using the B460 system. The drugs tested were isoniazid (INH), rifampin (RIF), streptomycin (SM) and ethambutol (EMB). Quality control using BACTEC

12B growth medium and AST was carried out using a standard H₃₇ Rv strain of MTB.

RESULTS

Thirty-eight males (76%) and 12 females (24%) were recruited, of which 10 males (26.3%) and 5 females (41.7%) were positive for TB. The other 35 cases were negative for AFB by both ZN staining and culture. The average age of females [median: 31; inter-quartile range (IQR): 30-37] was slightly younger than males (median 36; IQR 31-40). The majority of the patients in our study (88%) were married and from urban areas (60%). Commonest mode of transmission was heterosexual (80%). The CD4 cell counts ranged from 7 to 195 cells/ μ l with a mean of 124 ± 60 cells/ μ l (median: 124; IQR: 77 - 171 cells/ μ l). Thirty percent of cases belonged to the WHO HIV clinical stage II, 48% were stage III and 22% were stage IV.

Fifteen AIDS patients had a positive culture for MBT on BACTEC 12B vial medium; of which 6 were positive on L-J medium [sensitivity 40%, specificity 100%, positive predictive value (PPV) 100% and negative predictive value (NPV) 79.5%] and 5 on direct microscopy (sensitivity 33.3%, specificity 100%, PPV 100% and NPV 77.8%). The median time to yield a positive result with the B460 system was 14 days and with L-J medium was 28 days. Specimens with 2+ smear positive results with ZN smear yielded positive results by culture on L-J medium ($p=0.038$) and the B460 system ($p=0.007$) earlier than those that were smear negative.

MBT was found more often in patients with a PVL >4 log₁₀ copies/ml (OR: 4.6) than those with a PVL \leq 4 log₁₀ copies/ml (Table 1).

Table 1
Correlation between CD4 cell counts and the presence of *Mycobacterium tuberculosis*.

| Laboratory parameters | Test results (n) | No. (%) culture positive for MTB | Odds ratio (95% CI) | Median (IQR) |
|-----------------------------------|------------------|----------------------------------|---------------------|----------------------------|
| CD4 count(cells/μl) | ≤100 (21) | 8 (38.1) | 1.8 (0.58-6.4) | CD4 count: 124 (77-171) |
| PVL (log ₁₀ copies/ml) | 101-200 (29) | 7 (24.1) | 4.6 (8.1-22.5) | PVL: 5.1 (4.1 - >5.5) |
| | >4.0(18) | 8 (44.4) | | |
| | ≤4.0(13) | 2 (15.4) | | |

IQR, Interquartile range

PVL was only determined in 31 of 50 cases due to limited resources.

Table 2
Drug resistance patterns of *Mycobacterium tuberculosis* isolates against first-line anti-tuberculosis drugs (n=15).

| Observations | Total (%) |
|---|-----------|
| Total HIV positive cases | 50 |
| <i>M. tuberculosis</i> positive cases | 15 (30) |
| Sensitive to all drugs | 2 (13.3) |
| Resistance to any drug | 13 (86.6) |
| Resistance to one drug only | 8 (53.3) |
| INH | 6 (40) |
| RIF | 1 (6.7) |
| SM | 0 |
| EMB | 1 (6.7) |
| Resistance to more than one drug (excluding MDR TB) | |
| INH + SM + EMB | 1 (6.7) |
| MDR TB = combined resistance to at least isoniazid and rifampin | 4 (26.7) |
| INH + RIF + EMB | 2 (13.3) |
| INH + RIF + SM + EMB | 2 (13.3) |

INH, isoniazid; RIF, rifampin; SM, streptomycin; EMB, ethambutol

Of the 15 isolates, 8 (53.3%) had drug resistance to a single drug, 4 (26.7%) had multidrug resistance (MDR), and 1(6.7%) had resistance to 3 drugs (non-MDR) and 2 (13.3%) had no resistance to tested drugs (Table 2).

On the niacin and NAP test, all the isolates were found to be MBT.

DISCUSSION

In our study group, pulmonary TB due to MBT was found in 30% of AIDS patients, which is within the range seen in studies from India and other countries (Kaur *et al*, 1992; Nissapatorn *et al*, 2003; Praharaj *et al*, 2004). However, the percent

of pulmonary tuberculosis cases detected in AIDS patients in our study was on the lower side of the reported prevalence range (26-63%). This may be since our study group included late diagnosed HIV infected patients. The diagnosis of TB in such patients might have been delayed because of an atypical clinical presentation, a higher incidence of extra-pulmonary disease or disseminated disease leading to involvement of inaccessible sites and low sputum smear positivity (Sharma and Mohan, 2004).

The median CD4 cell count among the study group was found to be 97 cells/ μ l and the median PVL was 4.96 \log_{10} copies/ml. A study from Italy (Williams *et al*, 1999) found a median CD4 cell count of 144 cells/ μ l and a median PVL of 5 \log_{10} copies/ml among HIV-TB co-infected patients. The occurrence of MBT was more common among patients with a PVL >4 \log_{10} copies/ml (OR: 4.6) than in patients with a PVL \leq 4 \log_{10} copies/ml (Table 1). This is in accordance with a study from the USA (Williams *et al*, 1999) which found the viral load affected the occurrence of OIs, including TB, irrespective of the CD4 cell count, leading to development of OIs of varying frequencies in patients with similar CD4 counts.

Among the 15 isolates, monoresistance to RIF, SM and EMB was found in 6.7, 0 and 6.7% of isolates, respectively, similar to other studies from India and abroad (Ferdinand *et al*, 2003; Periera *et al*, 2005). Monoresistance to INH was seen in 40% of cases, which is much higher than reported in other studies (7 - 10%) (Ferdinand *et al*, 2003; Praharaj *et al*, 2004; Periera *et al*, 2005). The prevalence of multidrug resistance (resistance to at least INH and RIF) in our study group was 26.7%, compared to 4.3 - 10% reported

from other studies from India and abroad (Ferdinand *et al*, 2003; Praharaj *et al*, 2004; Periera *et al*, 2005). One study from Italy has found 36% of cases had MDR TB among new TB cases co-infected with HIV (Angarano *et al*, 1998). The prevalence of polyresistance (resistance to two or more drugs, but not both INH and RIF) was found to be 6.7% in our study subjects compared to 10% from another study from India (Periera *et al*, 2005).

Our study was limited by a number of factors; therefore, the results should be interpreted with caution. Our sample may not have been representative of the entire population of patients living with AIDS in the country because recruitment of respondents was done at a single tertiary healthcare facility. The main strength of the study results reflect the status of pulmonary tuberculosis and drug susceptibility patterns among HIV patients with a CD4 cell count <200 cells/ μ l and who are eligible for antiretroviral therapy. Our data reveal resistance to first line anti-tuberculosis drugs is high among MBT isolates from AIDS patients. Early detection and treatment with appropriate anti-tuberculosis drugs is essential.

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