

Incidence and Drug Susceptibility pattern of *Mycobacterium tuberculosis* in HIV infected Patients

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Abstract

Incidence of HIV infection in patients with tuberculosis was found out by serological method (ELISA) with confirmation by Western Blot analysis. Out of 2116 tuberculosis patients tested for HIV, 150 cases were found to be positive for HIV infection (5.73%). Drug susceptibility to first line antitubercular drugs was carried out in 1378 isolates from HIV negative cases and 68 isolates from AIDS cases. The overall resistance pattern to one or more drugs was seen in 13.78% of isolates from HIV negative cases as compared to 7.2% isolates in AIDS cases. Multidrug resistance (MDR) was seen in 8.9% of isolates from HIV negative cases as compared to 4.4% of isolates from AIDS cases.

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Key Words : Antibiotic susceptibility; HIV; Tuberculosis

Introduction

Tuberculosis (TB) remains one of the major public health problems in the world today. In the last decade, both developing and developed countries have experienced a startling increase in the incidence of TB as several factors have contributed to this upsurge. The single most important issue has undoubtedly been the emergence of the human immunodeficiency virus (HIV) pandemic. WHO has estimated over 88 million new TB cases with 30 million deaths in the present decade [1]. Up to 46% co-infection rates have been reported in developing countries [2]. In recent years, the treatment of TB has been threatened by increasing number of patients with MDR-TB, defined as resistance to isoniazid (INH) and rifampicin [3]. Though number of *M avium-intracellulare* complex (MAIC) remains the number one organism for TB in AIDS patients in western countries like USA, in India *M tuberculosis* (MTB) is the main causative organism [3]. The case fatality rate is high among patients with AIDS who are infected with strains of drug resistant *M tuberculosis* [4]. MDR-TB in AIDS patients is on the rise [5] and in future it may be alarming. This work was undertaken to find out the prevalence of HIV infection in patients suffering from TB and also to find in vitro drug susceptibility pattern to first line antitubercular drugs in the isolates of *M tuberculosis*.

Material and Methods

The study was conducted in a large service hospital. The study population consisted of 2616 patients with pulmonary and / or extra pulmonary TB aged between 5 to 72 years admitted to hospitals or undergoing OPD treatment. 250

healthy age and sex matched individuals were included as controls, to find out the sero-prevalence of HIV infection.

All the clinical samples obtained from patients were subjected to Ziehl Neelsen's (ZN) stain after concentration and culture on Lowenstein Jensen's (L-J) medium. Cultures were incubated for up to 12 weeks before being discarded as negative. Speciation of mycobacteria was carried out by standard methods [6]. All patients included in the study were screened for HIV-1 and HIV-2 antibodies in their sera using commercial ELISA kits (detect HIV Biochem Immune system Inc, Canada or Innostest HIV1/HIV2, Innogenetics, Belgium). All ELISA reactive sera were re-tested on a second EIA (Immunocomb, Biospot, Orgenics, Israel) and reactive sera were confirmed by Western Blot analysis.

A total of 1378 isolates of MTB from patients, without HIV infection and 68 isolates from patients with AIDS were subjected to antibiotic sensitivity using five first line drugs viz; streptomycin, isoniazid, rifampicin, pyrazinamide and ethambutol (SHRZE). In vitro drug susceptibility testing was performed by incorporation of required drug concentration and subsequent inoculation of L-J media with standardised inocula following the protocol of National Tuberculosis Institute, India [6]. Strains were declared resistant if growth of more than 20 colonies were observed at the following drug concentration :

Isoniazid	:	MIC of > 1 mg/L
Streptomycin	:	Resistance ratio >8
Rifampicin	:	MIC of >64 mg/L
Pyrazinamide	:	MIC of >100 mg/L
Ethambutol	:	MIC of >8 mg/L

Results

Over a period of five years a total of 2616 clinical samples from cases of TB were studied. The age wise distribution of

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HIV infection in TB patients is shown in Table 1. Out of 150 HIV positive patients, 139 (92.2%) were in the age group of 21-40 years. Only 1 out of 250 controls (0.4%) tested positive for HIV-1 infection. Overall sputum smear positivity for AFB was found in 524/2616 (20.03%) of cases of which 497/2466 (20.15%) were from HIV negative and 27/150 (18%) were from HIV positive patients. Total culture positivity for AFB was found in 1446/2616 (53.2%) cases (including smear positive cases). Out of total 1446 isolates of *M tuberculosis*, 68/150 (45.3%) isolates were from patients with AIDS and 1378/2466 (55.8%) from HIV negative cases (Table-2). All culture isolates were MTB. No atypical mycobacteria were grown.

Table 1
Age wise distribution of HIV seropositivity in TB patients

Age in years	Total tested	Total positive (%)
0-10	21	0 (0)
11-20	214	0 (0)
21-30	1031	41 (3.9)
31-40	842	98 (11.6)
> 41	508	11 (2.16)
Total	2616	150 (5.73)

Table 2
Smear and culture positivity of specimen

Status	Smear positivity (%)	Culture positivity (%)
HIV negative	497/2466 (20.15%)	1378/2466 (55.8%)
HIV positive	27/150 (18%)	68/150 (45.3%)
Total	524/2616 (20.03%)	1446/2616 (55.2%)

Sera from all 2616 patients of TB and 250 controls were screened for presence of HIV-1 and HIV-2 antibodies. 150/2616 (5.73%) sera from TB cases tested positive for HIV infection. One patient had dual infection with HIV 1 and HIV 2. Seropositivity of HIV infection was confirmed by Western Blot.

Out of 2616 cases, 2523 were of pulmonary TB alone. Rest included disseminated TB 53, meningitis 13, pleural effusion 18, TB lymphadenitis 5 and miliary TB 4 cases. Highest HIV positivity was seen in 17/53 (32.07%) cases of disseminated TB (Table 3).

Table 3
Incidence of HIV - TB co-infection in relation to the mode of clinical presentation

Clinical diagnosis	No. tested	No. positive (%)
Pulmonary TB	2523	127 (5.03)
Disseminated TB	53	17 (32.07)
Pleural effusion	18	01 (5.55)
TB meningitis	13	02 (15.38)
TB lymphadenitis	05	01 (20)
Miliary TB	04	02 (5)

In vitro drug susceptibility testing to first line antitubercular drugs (SHRZE) was carried out and the

resistance pattern is shown in Table 4. Overall resistance to one or more drugs was seen in 13.8% of isolates (190/1378) from HIV negative cases and in 7.3% (5/68) in AIDS cases. All resistant cases in both the groups showed resistance to streptomycin. INH resistance in combination with other drugs was seen in 146/1378 (10.6%) in HIV negative cases and in 5/68 (7.3%) of AIDS patients. Similarly, for rifampicin the figures were 154/1378 (11.2%) and 4/68 (5.8%) respectively. Four drug (SHRZ) resistance was seen in 3 isolates from HIV negative cases (data not shown in Table). MDR (R+H) was seen in 122/1378 isolates (8.9%) from HIV negative cases and 3/68 (4.4%) from AIDS cases (Table 4).

Table 4
Pattern of drug resistance in *M tuberculosis* isolates

Drug	HIV negative (N=1378)	HIV positive (N=68)
	Total no resistant (%)	Total no resistant (%)
Streptomycin	190 (13.78)	5 (7.3)
Isoniazid	146 (10.6)	5 (7.3)
Rifampicin	154 (11.2)	4 (5.8)
Pyrazinamide	133 (9.7)	2 (2.9)
Ethambutol	67 (4.8)	2 (2.9)
MDR (R+H)	122 (8.9)	3 (4.4)

Discussion

In Jun 1981, the first report was published on occurrence of opportunistic infections, which form a characteristic part of AIDS presentation [2, 3]. TB is a frequent cause of such infection and is the most common mode of presentation of this syndrome especially in India [7].

In our study, we found a prevalence of HIV-TB co-infection in 150/2616 (5.73%) cases. In India, the incidence of HIV infection among TB patients is 3.4% in Manipur [7], 5.02% in Pondicherry [8], 13.2% in Mumbai [9], 1.4% in Lucknow [10] and 30% in Calcutta [11]. Out of the 150 HIV positive cases 139/150 (92.2%) were from patients between the age group of 21-40 years, suggesting high prevalence rate in sexually active group. Out of the 53 disseminated TB cases, 17 (32.07%) were HIV positive suggesting that HIV positive patients are prone to disseminated tubercular infection. The overall smear positivity in our study was 524/2616 (20.03%) and similar findings were reported by other Indian studies [12]. Smear positivity was seen in 27/150 (18%) AIDS patients and 497/2466 (20.15%) in HIV negative cases (Table 2). The culture positivity was 45.3% in AIDS patients as compared to 55.8% in HIV negative cases (Table 2). It has been shown by other studies also that AFB culture positivity of sputum samples are lower in HIV infected individuals than in HIV negative cases [13,14]. The reasons attributed are the predominance of non-cavitating lesions and extra pulmonary TB in HIV infection [15]. In our case, this

could also be due to the fact that these patients were already on antitubercular treatment (ATT) when cultures were taken.

All the isolates were *M tuberculosis* and no non-tubercular mycobacteria (NTM) were isolated. MAIC is the leading causative organism causing TB in HIV infected patients in most of the western countries whereas in India, *M tuberculosis* is the commonest cause [3]. Overall resistance to one or more drugs was 13.78% with streptomycin resistance being the highest. Similar resistance to one or more drug has been reported by Jena et al. and Sonnenberg et al. who had found 12.7% and 11% respectively [16,17]. MDR defined as resistance to INH and rifampicin was seen in 8.9% cases. Similar observation was made by Jena et al. earlier when they had found MDR in 8.3% of isolates [16]. Incidence of MDR has been reported to 14.1% in Estonia, 10.8% in China, 10.8% in Iran [18] and 5.7% in South Africa [17]. In many countries, MDR cases are already on the rise and incidence ranging from 5.3% (New Zealand) to 100% (Ivanovoblast, Russian Federation) have been reported [5]. Surprisingly, isolates from the AIDS patients showed a different resistance pattern. The overall resistance to one or more drugs was seen in 7.2% of isolates from HIV positive cases as compared to 13.8% of isolates in HIV negative cases. Similarly, MDR was seen in only 4.4% isolates as compared to 8.9% of isolates from HIV negative cases. Lower incidence of MDR TB in HIV infected patients has also been reported by Sonnenberg et al [17]. This may be due to the fact that in AIDS patients, TB occurs due to reactivation and strains were sensitive to first line antitubercular drugs. This suggests that full course of ATT with first line drugs will be effective in most of the cases. The scenario may change after a few years due to irregular treatment resulting in secondary drug resistance.

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