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Study of HIV Seroprevalence in Pulmonary Tuberculosis Patients with Special Reference to Multidrug Resistant Mycobacteria

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ABSTRACT

This study was planned to determine HIV seroprevalence among pulmonary tuberculosis patients, to characterize the isolated mycobacteria into typical and atypical strains and to evaluate the drug resistant pattern of mycobacterial isolates. The study aims to correlate multidrug resistance (MDR) and HIV seropositivity status in pulmonary tuberculosis patients. During the year 1994-1997, 750 pulmonary TB patients were screened for the presence of anti- HIV 1 & 2 antibodies by commercially available kits of enzyme immunoassay (EIA). Sputum samples were screened for acid fast bacteria by Ziehl-Neelsen technique and isolated on Lowenstein-Jensen (LJ) media. Identification of *M. tuberculosis* was done by standard biochemical tests. Drug sensitivity testing was carried out using a standard inoculum onto LJ slopes containing different concentrations of 5 anti-TB drugs, namely isoniazid, rifampicin, ethambutol, streptomycin and ethionamide. Out of 750 patients screened, 177 (23.6%) were detected to be HIV seropositive (Group I) and 573 were seronegative cases (Group II). Among these 2 groups, 135 (76.27%) and 473 (82.55%) patients yielded mycobacterial cultures on LJ slopes respectively. Drug sensitive testing revealed 25.19% and 12.05% isolates to be resistant to all the 3 first line drugs namely, rifampicin, isoniazid and ethambutol in seropositive and seronegative groups respectively. From our study it is evident that multidrug resistance has shown a marked increase to the first line anti-TB drugs in HIV seropositive pulmonary tuberculosis patients. *Iran. Biomed. J. 3 (1 & 2): 15-21 1999*

Keywords : Drug resistance, HIV infection, Pulmonary TB, MDR-TB

INTRODUCTION

Tuberculosis is still the most important plague of mankind. The World Health Organization (WHO) estimated that 1.7 billion people have been infected with *M. tuberculosis*, and that each year 8 million people develop tuberculosis in recognizable form world-wide and 3 million die from it. This problem has been further aggravated due to wide spread Human Immunodeficiency Virus (HIV) infection.

The pandemic of HIV disease and its impact on tuberculosis (TB) require research in these two diseases. TB infection is the most frequent and serious complication of HIV infection. Risk of tuberculous infection progression to active disease is about 30 times higher in HIV seropositive individual than seronegative ones [1]. Hence, screening of TB patients for HIV infection becomes out most important.

HIV-TB link may have ominous implications for a country like India which harbors almost half the total number of TB cases in the world and where 50% populations over 20 years of age is infected with *M. tuberculosis* [2]. It is estimated that nearly 1.5 million HIV infected cases already prevail in India, majority of them being in Mumbai [2, 3] with rapid spread of HIV and tuberculosis infection, there appears to be an onset of a twin HIV-TB epidemic in the country.

Because of poverty, malnourishment, over-crowding, alcoholism, air pollution, smoking habits etc., management of spread of TB is very difficult and this problem is aggravated further due to emergence of multi-drug resistant (MDR) mycobacteria. Resistance to anti-tuberculosis drugs is a global problem [4]. The problem of resistance results from treatment that is inadequate, often because of an irregular drug supply, inappropriate regimes, or poor compliance. Center for Disease

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Control and prevention (CDC) in Atlanta has reported about a dozen of outbreaks of multidrug resistant TB (MDR-TB) in the USA, involving about 300 cases, where more than 90% of patients were HIV positive and some of the mycobacterial isolates were found to be resistant to as many of 7 anti-tuberculosis drugs [5, 6]. Resurgence of TB and a concurrent increase in the occurrence of *M. tuberculosis* resistant to isoniazid, rifampicin and other drugs has been cited as a global emergency [7]. Recent studies indicated that acquired resistance among treatment failures varied between 40-70% to isoniazid, 20-30% to rifampicin and 15-30% to streptomycin [8]. However, there are very few reports revealing the exact drug resistance pattern in HIV infected TB patients in India. AS the level of drug resistance pattern in a community is an epidemiological parameter, such data is essential to plan and access the success of the ongoing TB treatment program.

The present study was, therefore, undertaken to evaluate the drug resistance pattern of mycobacterial isolates from pulmonary TB patients and to determine HIV seroprevalence among these subjects.

MATERIALS AND METHODS

The study included 750 pulmonary TB patients diagnosed on the clinical and radiological evidences from Group of TB hospitals, Mumbai, during the year 1994-1997. Patients of both sexes and all ages were included. Informed consent was obtained from each subject case and their detailed clinical history with special reference to previous Koch's infection and or anti TB treatment, high risk behavior recorded.

Serum samples from these patients were screened for HIV 1 and 2 infections by commercially available of enzyme immunoassay (EIA). Initial screening was performed using Novapath HIV 1 and 2 EIA (Bio-Rad laboratories). The samples detected positive by initial screening were confirmed in duplicate by Abbott Recombinant HIV 1 and 2 EIA (Abbott diagnostics). Samples, which were reactive by both assays, were considered as HIV positive in the present study.

All the subject cases were instructed to expel a deep productive cough. Early morning sputum samples were collected in sterile wide mouth screw bottles for three consecutive days from all the patients and smears were examined for the presence of Acid-Fast bacilli following Ziehl-Neelsen staining [9]. Irrespective of smear positivity, all the sputum samples were concentrated by using 4.5% sodium lauryl sulfate (SLS) and 1.5% sodium hydroxide [10] and cultured on Lowenstein-Jensen (LJ) media slopes. Isolates obtained were identified by series of standard biochemical tests [11].

Drug sensitivity was carried out using a standard inoculum by resistance ratio method for isoniazid, rifampicin, ethambutol, streptomycin, ethionamide [12].

M. tuberculosis H37Rv was used as a control. An isolate was referred to MDR, if it exhibited resistance to two or more anti TB drugs tested. Data accrued from the above experiments were analyzed for statistical validity by chi square test with Yate's correction [13].

RESULTS

A total of 177 cases out of 750 pulmonary TB patients were found to be HIV seropositive (Group I) while the remaining 573 cases were HIV seronegative (Group II), thus accounting HIV seroprevalence rate of 23.6%. Highest HIV seroprevalence was observed in the sexually most active age group of 21-40 years with male (75.00%) and female (80.00%) percentages being comparable (Table 1).

Overall, 608 (81.07%) subjects yielded mycobacterial isolates. Table 2 correlates HIV seropositivity status of pulmonary TB cases with *in vitro* culture results. Among Group I, 135 (76.27%) were culture while in Group II, 473 (82.55%) were culture positive. Thus showing no significant difference between both groups (Chi square 0.008, $p>0.05$).

Among the 608 isolates 566 (93.09%) were typical *M. tuberculosis* strains, of which 111 (83.22%) were from Group I while 455 (96.19%) were from Group II. The remaining 42 (6.91%) were atypical strains, 24 (17.78%) belonging to Group I while 18 (3.81%) belonging to Group II. *M. kansasii* was the most frequent atypical isolate, followed by *M. scrofulaceum*. Among Group I, *M. avium* complex was the most frequent atypical strain isolated (Table 3).

Majority of subject cases gave history of anti-TB treatment for at least 6 months. 94.81% of Group I cases and 89.75% of Group II cases had taken anti Koch's therapy, with isoniazid and rifampicin. Streptomycin was the third most common drug used

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Table 1. Sex and agewise seroprevalence of HIV in TB patients (n=750). Figures in the parenthesis indicate percentages.

Sex	N	11-20 Yrs	21-30 Yrs	31-40 Yrs	41-50 Yrs	51 Yrs +
M	132	-	32	67	21	12
	(74.58)		(24.24)	(50.76)	(15.91)	(9.09)
F	45	-	18	18	9	-
	(25.42)		(40.00)	(40.00)	(20.00)	
Total	177	-	50	85	30	12
	(23.6)		(28.25)	(48.02)	(16.95)	(6.78)

Table 2. Correlation of HIV seropositivity and pulmonary tuberculosis confirmed by *in vitro* culture (n=750). Figures in the parenthesis indicate percentages.

HIV Serostatus	No. Of Samples		
	Studied	Culture + VE*	Culture - VE
Positive (group I)	177	135	42
		(76.27)	(23.73)
Negative (group II)	573	473	100
		(82.55)	(17.45)
Total	750	608	142
		(81.07)	(18.93)

* $X^2 = 0.008$ $p>0.05$.

Table 3. Incidence of atypical mycobacteria among pulmonary TB patients. Figures in parenthesis indicate percentages.

Isolates	HIV serostatus		
	Positive (n=135)	Negative (n=473)	Total (n=608)
Typical *M. tuberculosis*	111 (83.22)	455 (96.19)	566 (93.09)
Atypical	24 (17.78)	18 (3.81)	42 (6.91)
M. kansaii	8 (5.93)	10 (2.11)	18 (2.96)
M. avium complex	9 (6.67)	-	9 (1.48)
M. scrofulaceum	2 (1.48)	8 (1.69)	10 (1.64)
M. terrae complex	2 (1.48)	-	2 (0.33)
M. simiae	2 (1.48)	-	2 (0.33)
M. szulgai	1 (0.74)	-	1 (0.16)

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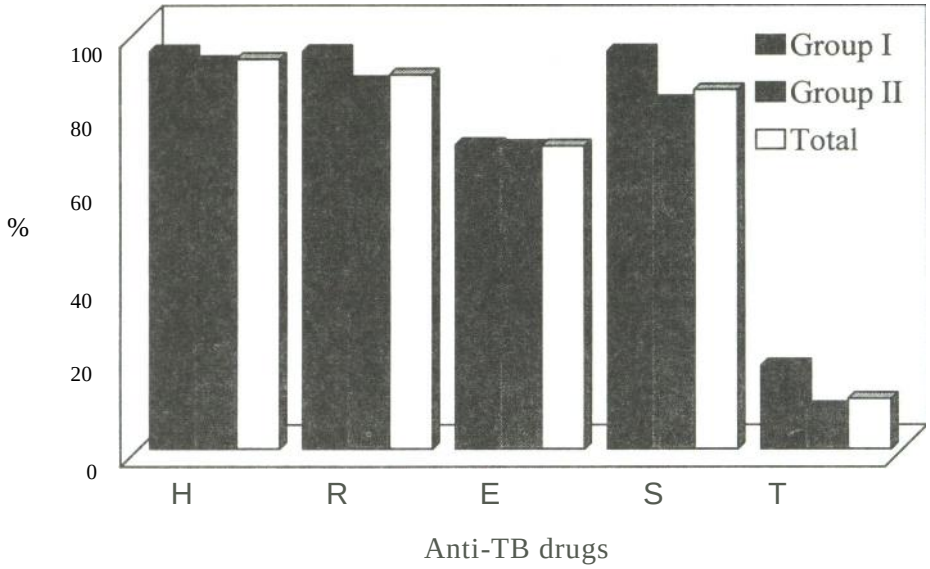

Fig. 1. History of previous anti-tuberculosis therapy (n = 608). Group I, HIV seropositive; Group II, HIV seronegative; H, isoniazid; R, rifampicin; E, ethambutol; S, streptomycin; T, ethionamide.

in anti-Koch's treatment regimen (Fig. 1). Drug sensitivity results are summarized in Tables 4 and 5. In both, HIV seropositive and seronegative groups highest resistance was observed for isoniazid (74.81% and 57.08% respectively) followed by rifampicin (65.19% and 41.86% respectively). Thirty four (25.19%) of Group I isolates and 57 (12.05%) of Group II isolates were resistant to 3 first line anti-TB drugs namely isoniazid, rifampicin and ethambutol. Likewise, in both these groups resistance to two drugs was most common and observed in 41 (30.37%) of HIV seropositive cases and 126 (26.64%) of HIV seronegative cases. Resistance to anti-TB drugs was found to be markedly higher in HIV seropositive patients (82.96%) than in HIV seronegative patients (72.30%) (chi square = 5.76 $p < 0.05$). Also, MDR was significantly higher in the former group (71.11%) as compared to the later group (46.30%) (chi square = 25.88 $p < 0.001$).

DISCUSSION

A rising trend has been observed in HIV seroprevalence among TB patients all over the world. Surveys in TB clinics in the U.S. revealed a median HIV seroprevalence rate of 3.4% with a range of 0-46% [14, 15]. In Sub-Saharan African countries HIV seropositivity ranged from 20% (Zaire) to 67% (Uganda) among TB patients [16, 17].

In India, also a number of studies have confirmed the rising HIV seropositive among TB patients from 0.39 in 1991 to 14.5% in 1994 [18.19]. Vyas A (1995) reported 22.72% HIV seropositivity among TB cases in Bombay [18]. A rate of 23.6% found in the present study confirms the higher HIV seroprevalence among TB patients.

Thus, it is evident that there is a rapid rise in the cases of dual infection of TB & HIV in Maharashtra, especially in Mumbai resulting from its overcrowding and floating population. There are an estimated 2-5 million people infected with HIV in India today, and 50,000 to 100,000 cases of Acquired Immune Deficiency Syndrome (AIDS) may have already occurred in the country. An estimated 1-2 million cases of tuberculosis occur in India every year. In Mumbai 10% of the patients presenting with TB are HIV positive. TB is the presenting symptom of AIDS in over 60% of AIDS cases [20]. Apart from the fact that Mumbai represents 5-10% of country's HIV infected population [3], the high HIV seroprevalence rate in this study may be in part due to the presence of maximum number of subject cases from the sexually most active age group of 21-40 years.

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Table 4. Distribution of mycobacterial isolates resistant to anti tuberculosis agents in relation to HIV status (n = 608). Figures in the parenthesis indicate percentages.

HIV Serostatus	NO. and % of mycobacterial isolates					
	Resistant to					Sensitive to
	H	R	E	S	T	HREST
Positive (Group I)	101	88	45	33	24	22
n=135	(74.81)	(65.19)	(33.33)	(24.44)	(17.78)	(16.30)
Negative (Group II)	270	198	75	85	28	130
n=473	(57.08)	(41.86)	(15.86)	(17.97)	(5.92)	(27.48)
Total	317	286	120	118	52	152
n=608	(61.02)	(47.04)	(19.74)	(19.41)	(8.55)	(25.00)
X^2	13.15	22.01	19.16	2.42	17.40	6.43
p Value	<0.01	<0.001	<0.001	<0.05	<0.01	<0.05

H=isoniazid, R=rifampicin, E=ethambutol, S= streptomycin, T=ethionamide.

Table 5. Incidence of drug resistance (figures in the parenthesis indicate percentages).

Isolates resistant to	No. and % of resistant isolates in	
	Group I (n=135)	Group II (n=473)
One drug only	16 (11.85)	124 (26.22)
Two drugs	41 (30.37)	126 (26.64)
Three drugs	34 (25.19)	57 (12.05)
Four drugs	6 (4.44)	15 (3.17)
All five drugs	15 (11.11)	21 (4.44)
2 or More drugs (MDR)*	96 (71.11)	219 (46.30)
1 or more drugs (Total resistance)**	112 (82.96)	342 (72.30)

Group I, HIV seropositive; Group II, HIV seronegative; *chi square = 25.88p<0.001, **chi square = 5.76p<0.05.

Atypical mycobacteria were uncommon cause of human disease. Their importance has increased in recent years because of their association with AIDS. MAC and M. kansasii frequently cause human disease [21]. MAC disease has become very common being diagnosed in 14-30% of HIV infected patients during life [22]. In the present study maximum number of isolates obtained was typical *M tuberculosis* strains (93.09%). The remaining were atypical strains (6.91%). Maximum isolates were of *M. kansasii*. Among HIV seropositive cases, maximum isolates were of *M. avium* complex.

In this study MDR was markedly higher in HIV seropositive TB patients than TB patient without HIV infection. As majority of cases had taken anti-TB treatment for at least 6 months, the acquired drug resistance is reflected in the study. In contrast, in Abidjan, Cote d'Ivoire, where newly diagnosed TB cases were studied, *M. tuberculosis* isolates from HIV positive and HIV negative patients did not differ significantly in primary resistance to anti-TB drugs. Also, the relatively smaller sample size in the Abidjan study might have been responsible for the ambiguous results [23]. On the other hand, a major increase in the resistance isolates of *M. tuberculosis* in HIV seropositive individuals has been reported in New York City [24].

Subject cases in this study have been selected from Group of TB hospitals, Mumbai, which is major referral center for chronic TB cases in India and majority of these patients have had anti-TB treatment for at least 6 months. This may be responsible for high multidrug resistance encountered in this study.

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Our findings confirm the higher HIV seroprevalence rate among TB patients and increased incidence of MDR strains among HIV infected TB cases. This emphasizes the need to keep a check in indiscriminate use of anti-TB drugs and advocates Directly Observed Therapy (DOT) to control the situation. The idea of targeting isoniazid preventive therapy to individuals with dual infection should be considered as an important intervention that may reduce the impact of HIV associated TB in the long term. Public health services will have to strengthen their resources and tuberculosis control program should be linked with AIDS prevention and control policy to achieve better results. Detailed studies are essential to monitor the TB-HIV dual epidemic over an extended period.

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