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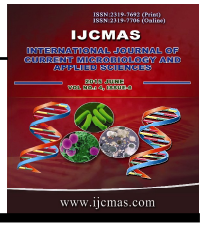

Original Research Article

Prevalence of Pulmonary Tuberculosis in HIV Positive Individuals, Its Sensitivity and Association with CD4 Count

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A B S T R A C T

There is grave concern regarding increase in HIV associated Tuberculosis (TB) and emergence of Multi Drug Resistant (MDR) and Extremely Drug Resistant (XDR) TB. It is essential to know prevalence of TB in HIV patients, its sensitivity and association with CD4 count. A total of 362 patients were screened for Pulmonary TB of which 85 (23.48%) were diagnosed as Pulmonary TB by radiology, Ziehl Neelsen (ZN) smear and culture. Drug susceptibility testing was done to find out whether the organisms isolated in a culture was sensitive or resistant to anti-TB drugs used for the treatment of a patient. The tests were performed according to the standard methods.CD4 counting of blood samples were done by Flow cytometry. Correlation of CD4 cell counts was done with the pulmonary tuberculosis in HIV positive patients. The results of the study emphasize that co-infection of TB in HIV/AIDS patients is a concern. There is direct correlation between CD4 counts depletion and Pulmonary TB in HIV/AIDS patient. Hence, regular monitoring of these patients is warranted.

Introduction

AIDS, the Acquired Immunodeficiency Syndrome, is the disease known to be scourge for our century has had an impact like no other disease. It has reflected with its spread, the spread of immorality, sexual freedom and ignorance. Acquired Immunodeficiency Syndrome (AIDS) is the terminally fatal stage of Human Immunodeficiency Virus (HIV) infection, characterized by a variety of symptoms and signs predominantly due to bacterial, viral, protozoal and fungal infections because of reduction in body's immune defence mechanism, as a result of depletion of helper T lymphocytes. Although reports of the HIV epidemic emerged from the developed and industrialized countries initially, now focus is shifting fast to south East Asia in which India contributes to a major bulk of cases (WHO, 2003). HIV affects the human Helper T lymphocytes and macrophages, which are important in maintaining Cell

Mediated Immunity (CMI). HIV is the most important known risk factor that promotes progression to active tuberculosis in people with Mycobacterium tuberculous infection (WHO, 2004). Tuberculosis (TB) and HIV have been closely linked since the emergence of AIDS. The lifetime risk of tuberculosis in immunocompetent persons is 5% to 10%, but in HIV positive individuals, there is a 5% to 15% annual risk of developing active TB disease (Swaminathan *et al.*, 2000). The patterns of clinical presentation of TB depend on the host immune status which is reflected in the microbiological, radiological and histological characteristics of TB.

There is a close relationship between clinical manifestations of HIV infection and CD4+ T cell count which has made measurement of the latter a routine part of the evaluation of HIV-infected individuals. The CD4+ T cell count is the best indicator of the immediate state of immunologic competence of the patient with HIV infection. The appearance of many opportunistic infections correlates with the CD4 count. TB generally develops at CD4 counts of 200-500 cells/mm3. Thus determinations of CD4 cell counts provide a powerful set of tool for determining prognosis and monitoring response to therapy (Fauci *et al.*, 2008).

Therefore the present study has been undertaken to know the prevalence of PTB in HIV positive individuals, to diagnose pulmonary tuberculosis in HIV positive individuals using conventional staining methods (Ziehl Neelsen stain), culture (Lowenstein–Jensen) and radiological evidence, to identify the species of mycobacteria by different biochemical tests, to study the prevalence of pulmonary tuberculosis in HIV positive individuals,to determine the antituberculosis drug sensitivity pattern of these isolates, to study the correlation of pulmonary tuberculosis with the CD4+ cell counts in HIV positive individuals.

Material and Methods

The Prospective Hospital Based Study was conducted in the Department of Microbiology, from November 2007 to October 2009 in association with the ART clinic, Medicine, Integrated Counseling & Testing Centre, Chest and TB wards of our hospital. A total of 362 known HIV positive patients of 18 years of age and above either hospitalized or coming to ART clinic, clinically suspected of having pulmonary tuberculosis, after written informed consent, were included in the study. The patients with extra pulmonary manifestations were excluded from the study. Two sputum samples were obtained from each patient as per RNTCP guidelines. Smears were prepared and stained by Ziehl Nielsen stain. The culture was done on Lowenstein-Jensen medium (LJ) The growth was identified by standard methods (AIDSCAP, 1996).Drug susceptibility testing was done to find out whether the organisms isolated in a culture was sensitive or resistant to anti-TB drugs used for the treatment of a patient. The tests were performed according to the standard methods. About 3 ml of blood was collected from each patient using aseptic precautions in EDTA vacutainers. CD4 counting of blood samples was done by Flow cytometry as per manufacturer's instructions (FACS Calibur, Becton- Dickinson, Immuno-cytometry system). The findings of the chest X-ray were noted of each patient. Correlation of CD4 cell counts was done with the pulmonary tuberculosis in HIV positive patients.

Observation and Results

A total of 362 HIV positive patients clinically suspected of having Pulmonary Tuberculosis were included in the study. Out

of 362 HIV positive patients, 28 (7.73%) sputum specimens were positive on both microscopic examination (ZN stain) and culture. However out of the total 34(9.39%) culture positive cases, 6 sputum specimens were negative by the ZN stain but were culture positive. The remaining 328 (90.61%) sputum specimens were negative by both ZN stain and culture (Table 1). Amongst these 362 patients, culture positive PTB cases were 34(9.39%). There was radiological evidence in 51(14.09%) cases which was strongly suggestive of PTB, even though they were negative by ZN smear. These cases were treated with the antibiotic course, and were ZN negative even on repeat sputum examination. And hence were included as the radiologically positive PTB cases. Thus the total no. of PTB diagnosed cases were 85(23.48%) (Table 2).

The sputum samples of all 362 HIV positive patients clinically suspected of having PTB was cultured on LJ medium and only 34(9.39%) of these patients showed the growth on LJ medium. Out of these 34 mycobacterium isolates, growth of only 33 (97.06%) isolates appeared within 2-3 weeks of incubation and was confirmed as slow growers and only 1 (2.86%) isolate showed the growth within 7 days and was confirmed as rapid grower.

The colonial morphologies of all 33(97.06%) isolates were dry and rough with irregular margins, typical eugonic buff growth without any pigmentation whereas only 1(2.86%) isolate showedcolonies which was smooth, butyrous or waxyin consistencyand waspigmented (orange). The identification of these isolates was further confirmed by the biochemical assays as shown in (Table 3). The 33 (97.06%) of these isolates were confirmed as Mycobacterium tuberculosis from there characteristic growth rate, colonial morphology on LJ medium and by biochemical assays likeNitrate reduction testas well as Niacin accumulation test which was positive and Para-Nitro benzoic acid susceptibility test which was negative. Only 1(2.86%) isolate was confirmed as NTM (Nontuberculous Mycobacteria) and was identified as Rapid grower from their growth rate, pigmentation, colony characteristics and by biochemical assays as shown in (Table 3).

Drug sensitivity tests were performed by incorporating required drug concentration of antimycobacterial drugs in the LJ medium. Control strain, H37RV, was set up with each batch for drug sensitivity testing and the strains were defined resistant, if more than 20 colonies were obtained on drug slant media. Resistance ratio (RR) method of drug sensitivity was carried out for Streptomycin (S) and minimum inhibitory concentration (MIC) for Isoniazid (H), Rifampicin (R), Ethambutol (E) by Absolute Concentration Method (Table 4).

Control strain, H37RV, was set up with each batch for drug sensitivity testing for all the four drugs and their concentration mentioned in the above table. Resistance ratio (RR) method of drug sensitivity was carried out for Streptomycin (S) and it was found to be sensitive. The H37RV strain was tested for Isoniazid (H), Rifampicin (R) and Ethambutol (E) by Absolute Concentration Method and was found to be sensitive to all of them (Table 4).

Drug susceptibility pattern of 34 Mycobacterial isolates to isoniazid

Drug susceptibility of 34 Mycobacterial isolates to isoniazid was tested by Absolute concentration method as mentioned in the Manual on isolation identification and sensitivity testing of mycobacterium

tuberculosis, National Tuberculosis Institute, 1998.For doing the sensitivity tests, the mycobacterium isolates were exposed to a series of appropriate concentrations of each drug. The three concentration of isoniazid used was 0.2 µg /ml, 1 µg /ml and 5 µg /ml. Out of 34 mycobacterial isolates 31(91.18%)showed no growth in 0.2 / µg ml slope and 3 (8.82%)showed growth on 1 or more than1µg /ml. Thus interpretation of sensitivity test result was done as per standard guidelines. 31 (91.18%) isolates were sensitive to isoniazid and 3 (8.82%) were resistant to it.Doubtful resistance was not shown by any isolate (Table 5).

Drug susceptibility of 34 Mycobacterium isolates to streptomycin was tested by resistance ratio method.

The two concentration of streptomycin used was 16 µg /ml and 32 µg /ml. The resistant ratio was determined as mentioned in Annexure no.7. Out of 34 mycobacterium isolates, 33 showed resistance ratio less than 4 and only 1 isolate showed resistance ratio 8 and above. Thus interpretation of sensitivity test result was done as per standard guidelines. 33 (97.06%) isolates were sensitive to streptomycin and 1 (2.94%) was resistant to it.Doubtful resistance was not shown by any isolate. (Table 6)

Drug susceptibility of 34 Mycobacterium isolates to Rifampicin was tested by absolute concentration method.

The three concentration of Rifampicin used was 32 µg /ml, 64 µg /ml and 128 µg /ml. The Minimum Inhibitory Concentration (MIC) was determined. Out of 4 mycobacterium isolates 29(85.29%) showed MIC less than 64 and 5 (14.71%) showed MIC 64 and above. Thus interpretation of sensitivity test result was done as per standard guidelines. 29 (85.29%) isolates were sensitive to Rifampicin and 5 (14.71%) were resistant to it (Table 7).

Drug susceptibility of 34 Mycobacterium isolates to Ethambutol was tested by Absolute concentration method. The three concentration of Ethambutol used was 4 µg /ml, 5.6 µg /ml and 8 µg /ml. The Minimum Inhibitory Concentration (MIC) was determined. Out of 34 mycobacterial isolates 31 (91.18%) showed MIC less than 8 and3 (8.82%) showed MIC 8 and above. Thus interpretation of sensitivity test result was done as per standard guidelines. 31 (91.18%) isolates were sensitive to Ethambutol and 3 (8.82%) were resistant to it (Table 8). Antibiotic susceptibility pattern of 34 mycobacterium isolates showed resistance to two and three or more drugs in 4(11.76%) and 1 (2.94%) patients respectively. There was no single drug resistance seen. The double drug resistance was to H and R in 2 (5.88%) followed R and E in 2 (5.88%) cases each. MDR (H & R) was present in 3 (8.82%) cases (Table 9).

33 (97.06%) isolates were sensitive to streptomycin, 31 (91.18%) were sensitive to Isoniazid and Ethambutol each. 29 (85.29%) was sensitive to Rifampicin. Rifampicin revealed the highest resistance pattern in combination with other drugs in 5 (14.71%), followed by H in 3(8.82%), E in 3 (8.82 %) and S (2.94%) (Table 10).

In the present study, out of 85 HIV/PTB co infected patients, $CD4^+$ cell count of 48 (56.47%) cases was less than 200 $CD4^+$ cells/ µl, followed by 28 (32.94%) cases with $CD4^+$ count ranging from 200 to 349 cells/µl, 6 (7.06%) cases with 350–500 cells/ µl while 3 (3.53%) had above 500 $CD4^+$ cells/ µl. Thus PTB among HIV positive patients was more commonly seen when CD4 count was less than 200 cells/ µl. (Table 11).

AIDS is a pandemic of 21st century presenting with severe immunodeficiency in which patients present with symptoms of different opportunistic infections.HIV presently accounts for the highest number of deaths attributable to any single infective agent. India has an estimated 20.3 million HIV-infected people by the end of 2007 (UNAIDS, 2008). The threat to their life is not from the virus alone but by the different opportunistic infections (OI's) and associated complications with it. Pulmonary tuberculosis is the most common opportunistic infection (AIDSCAP, 1996). Thus it is very important to identify PTB at an earliest so that it can be managed appropriately.

In the present study 362 HIV positive patients clinically suspected of having PTB were included, to know the prevalence of PTB and its correlation with CD4 count.

The prevalence of PTB in HIV

Co-infection of TB has been a major concern in HIV/AIDS patients. TB remains an important public health problem and has been exacerbated by the HIV epidemic, resulting in increased morbidity and mortality worldwide. HIV-TB co-infection is "Bidirectional and synergistic" and is often designated as "Cursed Duet". HIV/AIDS leads to immunosuppression and is a strongest of all known risk factors for the development of TB. Thus in the present study, the prevalence of PTB was 23.48 % which was similar to Dhungana *et al.* (2008) who reported the prevalence of PTB 23%. Yanamadala *et al.* (2002) reported the prevalence to be 24.78%. The prevalence of co-infection with HIV varies widely across regions as shown in different studies mentioned above within India and outside India mainly due to the variation in the distribution of risk factors, geographic location, awareness levels etc of the study population. Amongst the Indian studies mentioned above the higher prevalence has been reported by Saini *et al.* (2004), Maniar *et al.* (2006) and Chakraborty *et al.* (2008) who reported prevalence to be 34%, 42% and 39.5% respectively, whereas Praharaj (2004) and Mahajan *et al.* (2008) reported the lower prevalence as 5.03% and 7.39% respectively. The lower prevalence of PTB can be attributed to the early diagnosis; increasing awareness and high index of suspicion about the presence of TB and timely prophylaxis.

Characterization of 34 *Mycobacterium* isolates

In the present study 34(9.39%) of these HIV positive patients showed the growth on LJ medium. 33(97.06%) of these isolates were confirmed as MTB and only 1(2.86%) isolate was confirmed as NTM (Non tuberculous Mycobacteria). In the present study only single case of Non tuberculous Mycobacteria was reported. This was comparable to other studies in which MTB was more commonly isolated than NTM. Praharaj *et al.* (2004) and Zuber Ahmad *et al.* (2005) found 100% of the isolate to be MTB and NTM was not reported by them. Similarly, Maniar *et al.* (2006) reported 84% of Mycobacterium tuberculosis complex and 16% of NTM. Matee *et al.* (2008) during their study found 79.23% of mycobacterium isolates to be MTB and 20.77% to be NTM.

Drug susceptibility testing of 34 *Mycobacterium* isolates

Drug sensitivity tests were performed by incorporating required drug concentration of antimycobacterial drugs in the LJ medium. Resistance ratio (RR) method of drug sensitivity was carried out for Streptomycin (S) and minimum inhibitory concentration (MIC) for Isoniazid (H), Rifampicin (R) and Ethambutol (E).

Amongst the 34 mycobacterium isolate isolated in the present study, 97.06% isolates were sensitive to streptomycin, 91.18% were sensitive to Isoniazid and Ethambutol each. 85.29% was sensitive to Rifampicin.

14.71% isolates were resistant to Rifampicin, 8.82% were resistant to Isoniazid, 8.82% were resistant to Ethambutol and 2.94% were resistant to Streptomycin.

Antibiotic susceptibility pattern of 34 mycobacterium isolates showed resistance to double and three or more drugs in 11.76% and 2.94% patients respectively. There was no single drug resistance seen. The double drug resistance was to H and R in 2 5.88% followed R and E in 2 (5.88%) cases each. MDR (H & R) was present in 8.82% cases.

Estimates of initial drug resistance carried out at the TRC, Chennai by Paramasivan (1998) reported that primary resistance to isoniazid was 15.0 % to streptomycin 11.8 %, and to both isoniazid and streptomycin was 7.7 % during the period 1993–1996. Kimerling *et al.* (2002) reported resistance to H was seen in 10 %, to S in 7% to both the drug (H+S) in 5%. No rifampicin resistance was found and no MDR-TB was identified. Praharaj *et al.* (2004) found that the isolates resistant to Streptomycin was 7.3%, Isoniazid (7.3 %), Rifampicin (5.8 %), Pyrazinamide (2.9 %), Ethambutol (2.9 %) and MDR was seen in 4.4%. Swaminathan *et al.* (2005) reported the prevalence of drug resistance among the 204 HIV /TB co infected patients was 15.7% to H, 2.5% to E, 8.3% to S and 6.9% to R, either alone or in combination with other anti-tuberculosis drugs. MDR-TB was seen in 5.9%. Pereira *et al.* (2005) found that of the 30 isolates from HIV infected patients, 10 % were resistant to isoniazid (H), and 6.6 % to streptomycin (S), 6.6 % to ethambutol (E) and 10 % were MDR. Resistance to rifampicin was not observed. Chand *et al.* (2006) studied the incidence of drug resistance and pattern of susceptibility to antitubercular drugs in PTB and found 18.14% strains were resistant to Streptomycin, 4.65% to Rifampicin, 6.40% to Isoniazid, 0.58% to Pyrazinamide and 1.16% to Ethambutol. MDR was observed in 2.91% cases, of which resistance to Isoniazid and Rifampicin was present in 1.16% and their combination with other drugs in other 1.74% isolates. Haar *et al.* (2007) found MDR TB in 1.6%.Wolfart *et al.* (2008) found that resistance to one or more first-line anti-TB drugs was found in 71 (17.8%) of patients and the highest resistance rates were found to isoniazid (9.9%). Multidrug-resistant TB was found in 2.0% patients. Ngowi *et al.* (2008) studied PTB among people living with HIV/AIDS attending care and treatment in rural northern Tanzania and found that most of the Mycobacteria isolated from these patients were susceptible to Rifampicin, Streptomycin, Isoniazid and Ethambutol. Resistance to isoniazid was seen in only one patient (5%) and no cases of MDR were detected.

Correlation of pulmonary tuberculosis with $CD4^+$ cell counts

The appearance of many opportunistic infections (OI's) correlates with the $CD4^+$ cell count. In the present study out of 85HIV/Pulmonary tuberculosis co infected patients, $CD4^+$ cell count of48 (56.47%) cases was less than 200 $CD4^+$ cells/ µl, followed by 28 (32.94%) caseswith $CD4^+$count ranging from 200-349 cells/µl, 6 (7.06%) cases with 350-500 cells/ µl while 3 (3.53%) had above 500 CD^+_4cells/ µl. Thus PTB was more commonly seen when CD4 count was less than 200 cell/µl. Similar correlation was reported by other Indian and

foreign studies. Markowitz *et al.* (1997) in a prospective cohort study diagnosed active TB in 31 (2.74%) out of 1130 HIV positive patients and found that TB occurredmore frequently in persons with CD4 cell counts of less than 200 /ul. Lee *et al.* (2000) performed a study to evaluate the impact of HIV infection on the clinical presentation of TB. Most were in the advanced stage of HIV infection; 93% had CD4 cell count less than 200 cells/mm^3 concerning the site of TB involvement, they found that 37% had pulmonary involvement alone. Vajpayee *et al.* (2003) did a retrospective study in 421 HIV infected patients to document the characteristic OI of HIV-infected North Indian patients along with their CD4+ counts. In their study, tuberculosis was seen in 47% with mean CD4 count of 189 cells/µl. Veeranoot *et al.* (2003) in a study of 419 HIV/AIDS patients found development of AIDS defining illnesses only in 282 patients. Out of these 282, they reported TB in 48%. 40% of these patients had PTB with CD4 cell count < 200 cells/mm^3. Attili *et al.* (2005) found that TB was the commonest opportunistic disease. Pulmonary and extra pulmonary TB had low positive predictive value (PPV) (51% and 42%) for CD4 levels of <200 when compared with the disseminated form (specificity 87% and PPV 75%). Chakraborty *et al.* (2008) studied 125 HIV positive patients and found that patients with HIV positive PTB had median CD4 count of 105 cells/ mm^3 blood. Ngowi *et al.* (2008) studied PTB among people living with HIV/AIDS attending care and treatment in rural northern Tanzania and found PTB in 20/233 patients (8.5%). Amongst them 6 (30%) had CD4 count <200 Cells/mm^3, 7 (35%) had CD4 count 200–349 Cells/mm^3, 4 (20%) had CD4 count 350–500 Cells/mm^3 and 3 (15%) had CD4count >500 Cells/mm3. The Mean CD4+ T cells were 277.9.

In the 85 HIV/PTB co infected cases, the correlation between the year of seropositivity with CD4 count was done. 22 (25.88%) cases were seropositive from year 2005, and amongst them 15 (68.18%) cases had CD4 count < 200 cells/ µl, 5 (22.73%) had CD4 count between 200 and 349 cells/ µl. Thus almost 20 (90.91%) of these cases had CD4 count < 350 cells/ µl. Similarly 24 (28.24%) cases who were seropositive from the year 2006, 14 (58.33%) and 6 (25%) cases had CD4 count <200 cells/ µl and 200-349 cells/ µl respectively. Thus 20 (83.33%) cases had CD4 count < 350 cells/ µl. Similar findings were noticed in the cases who were seropositive from 2007, amongst these cases 11(44) had CD4 count < 200 cells/ µl and 200-349 cells/ µl each, and thus accounting to 22(88%) cases who had CD4 count < 350 cells/ µl. Similarly the patients who were seropositive in the recent year (2008 & 2009), all had CD4 count< 350 cells/ µl. This clearly shows that once the person is tested HIV seropositive, and as the duration increases, the chances of decrease in its CD4 count and the susceptibility of acquiring PTB increases. Similarly in present study, the patients who were seropositive in the recent year (2008 & 2009) had CD4 count< 350 cells/ µl and this signifies the fact that once the patient is infected with PTB, there is greater chance of gradual decrease in the CD4 count. This has been very well documented by other studies, Swaminathan *et al.* (2008) reported that as HIV progresses, there is cutaneous anergy as well as impaired tissue containment of Mycobacteria leading to widespread dissemination of Mycobacteria.

Table.1 Correlation between ZN staining and culture (n=362)

		Culture onLJmedium		Total(%)
		+	–	
ZN stain	+	28	-	28 (7.73)
	-	6	328	334 (92.27)
	Total (%)	34 (9.39)	328 (90.61)	362 (100)

Variable Value 95% Confidence Interval
Sensitivity0.82350.6543 to 0.9324
Specificity1.0000.9888 to 1.000
Positive Predictive Value1.000 0.8766 to 1.000
Negative Predictive Value 0.9820 0.9613 to 0.9934

Table.2 Pulmonary tuberculosis in HIV positive patients (n=362)

Pulmonary tuberculosis	No. of cases	Percentage (%)
Culture positive TB	34	9.39
Radiologicalpositive TB	51	14.09
Total	85	23.48

Table.3 Characterization of 34 Mycobacterium isolates

Strain	No (%)	Rate of growth	Pigmentation	Nitrate Reduction test	Niacin Accumulation test	*PNB test
M. tuberculosis	33 (97.06)	2-3 wks (slow growers)	No pigmentation	Positive	Positive	Negative
NTM (Nontuberculous Mycobacteria)	1 (2.86)	5 days (rapid grower)	Orange pigmentation	Negative	Negative	Positive

* Para-Nitro benzoic acid susceptibility test

Table.4 Drug sensitivity testing of Control strain H37RV at different concentrations of antibiotics

Name of the drug	**Concentration of drugs (µg /ml)**	**Results**
Isoniazid	0.05, 0.1, 0.2	Sensitive
Streptomycin	2.0, 4.0, 8.0	Sensitive
Rifampicin	4.0, 8.0, 16.0	Sensitive
Ethambutol	1.4, 2.0, 2.8	Sensitive

Table.5 Drug susceptibility pattern of 34 Mycobacterium isolates to Isoniazid

Antibiotic (concentration of antibiotic µg/ml) Isoniazid (0.2, 1, 5)	**Sensitive (%)**	**Doubtful Resistance (%)**	**Resistance (%)**
No of isolate showingno growth in 0.2 µg /ml slope	31 (91.18%)	-	-
No of isolate showing growth in 0.2 µg /ml only.	-	-	-
No of isolate showing growth on 1 or more than 1 µg /ml.	-	-	3 (8.82%)
Total	31 (91.18%)	-	3 (8.82%)

Table.6 Drug susceptibility pattern of 34 Mycobacterium isolates to Streptomycin

Antibiotic (concentration of antibiotic µg/ml) Streptomycin (16.0, 32.0)	**Sensitive (%)**	**Doubtful Resistance (%)**	**Resistance (%)**
No of isolate showing R.R of < 4.	33 (97.06%)	-	-
No of isolate showing R.R of 4.		-	-
No of isolate showing R.R of 8 and above.	-	-	1 (2.94%)
Total	33 (97.06%)	-	1 (2.94%)

Resistant Ratio (R.R)]. R.R = $\frac{\text{MIC of wild strain}}{\text{MIC of H37RV}}$

Table.7 Drug susceptibility pattern of 34 Mycobacterium isolates to Rifampicin

Antibiotic (concentration of antibiotic µg/ml) Rifampicin (32.0, 64.0, 128.0)	**Sensitive (%)**	**Resistance (%)**
No of isolates showing MIC less than 64	29 (85.29 %)	-
No of isolates showing MIC 64 and above	-	5 (14.71%)
Total	29 (85.29 %)	5 (14.71%)

Table.8 Drug susceptibility pattern of 34 Mycobacterium isolates to Ethambutol

Antibiotic (concentration of antibiotic µg/ml) Ethambutol (4.0, 5.6, 8.0)	**Sensitive (%)**	**Resistance (%)**
No of isolates showing MIC less than 8	31 (91.18%)	-
No of isolates showing MIC 8 and above	-	3 (8.82%)
Total	31 (91.18%)	3 (8.82%)

Table.9 Pattern of drug resistance (n=34)

Resistance to	**Resistance pattern**	**No of strains**
Single Drug	--	--
Two Drugs	R + E	2 (5.88%)
	H + R	2 (5.88%)
More than Three drugs	H+R+E+S	1 (2.94%)
Total	-	5 (14.71%)

Table.10 Drug susceptibility pattern of 34 Mycobacterium isolates

Antibiotic	**Sensitive (%)**	**Resistant (%)**
Isoniazid	31 (91.18%)	3 (8.82%)
Rifampicin	29(85.29%)	5 (14.71%)
Streptomycin	33(97.06%)	1(2.94%)
Ethambutol	31(91.18%)	3(8.82%)

Table.11 Correlation of Pulmonary Tuberculosis with CD4+ count in HIV positive patients (n=85)

$CD4^+$ count (cells/µl) range	**Pulmonary tuberculosis**	**Percentage (%)**
<200	48	56.47
200-349	28	32.94
350-500	6	7.06
>500	3	3.53
Total	85	100

In patients with latent TB Infection, the risk of developing active disease is higher among recently infected persons compared to those with chronic infection but is several hundred-fold higher among persons who acquire HIV.

Further, Lawn et al 2002[31] reported that among persons with HIV infection, newly acquired TB infection can rapidly progress to active disease. It is clear that HIV infection acquired after TB infection is a significant risk factor for development of active TB is mainly due to its effects on the immune system. HIV is associated with decreased chemo taxis, defective granuloma formation and maintenance, impaired antigen processing and presentation as well as generalized loss of CD4 T cells and selective clonal depletion of MTB specific CD4 T lymphocytes. While TB can develop at any CD4 count, extra pulmonary and disseminated forms of the disease are more common as immunodeficiency increases.

Thus, the results indicate that TB is a useful clinical indicator and one of the most profitable to be discussed in the progression of HIV infection with severe immunosuppression. Hence, it is still feasible to monitor CD4 cell count as one of the prognostic markers for HIV/AIDS patients prior to develop more complicated and life threatening conditions.

The prevalence of pulmonary tuberculosis in HIV positive patients definitely indicates early diagnosis, high level of suspicion and effective and aggressive treatment of HIV-TB co-infection. Resistant cases in HIV/PTB co-infected patients should be identified by culture and drug sensitivity testing as early as possible in order to prevent the emergence as well as spread of MDR-TB. HIV positive patient with lowCD4 count should be regularly monitored for PTB, so that PTB can be diagnosed at an earliest stage and further morbidity and mortality due to PTB and its complication (MDR TB, XDR TB, Extra PTB) can be prevented. Thus, it should be mandatory to screen every HIV/AIDS patient for TB co-infection and vice versa.

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