

# Round Table Conference Series

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HIV and Tuberculosis : Co-infection



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# HIV and Tuberculosis : Co-infection

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## 7. Reactivation of *Mycobacterium tuberculosis* as evidenced by fingerprinting

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Tuberculosis (TB) has remained in the forefront among all the infectious diseases and it has been responsible for a high rate of mortality and morbidity around the world especially in the developing countries. The co-existence of HIV/TB has substantial impact on global epidemiology of TB. The identification of specific strains involved in the spread of infection gains great significance owing to drastic changes in the global epidemiology of TB.

TB is the most common opportunistic infection in people living with HIV. As the Human immunodeficiency virus (HIV) breaks down the immune system, HIV infected people are at increased risk of TB. HIV infection is the single strongest risk factor for progression of TB infection to TB disease. Without HIV, the lifetime risk of developing TB disease in TB infected people is 10% but the annual risk of TB infection in HIV patients is 10%. *Mycobacterium tuberculosis* accelerates progress of HIV to AIDS.

Tuberculosis disease develops in an individual due to progression of latent or primary infection, exogenous reinfection or endogenous reactivation. It is estimated that one third of the world's population is latently infected. Approximately 5-10% of the latently infected individuals may subsequently develop active tuberculosis during their life time. Host and microbial factors play a role in maintenance and progression of latent primary infection.

Even after the patients have undergone complete treatment they can come down with tuberculosis disease again. In the past it was difficult to understand whether the patient has relapsed due to exogenous reinfection or endogenous reactivation. With the availability of molecular tools it is possible to identify the exogenous infection from endogenous reactivation. Controversy prevails regarding the relative importance of endogenous reactivation and exogenous reinfection as the cause of recurrent tuberculosis. Monitoring the control of tuberculosis by epidemiological information is very important especially in HIV/TB patients who tend to acquire reinfection frequently because of their weak immune system. Molecular epidemiology can help in identifying exogenous reinfection and this data combined with drug susceptibility results would accelerate the treatment of HIV/TB patients with effective chemotherapy.

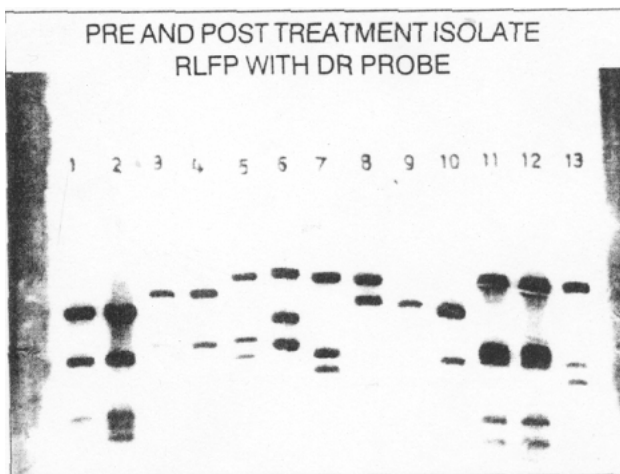
Restriction Fragment Length Polymorphism (RFLP) with the widely reported IS6110 probe has been considered an excellent tool. But the RFLP study conducted at TRC, South India using IS6110 revealed that 40% of south India strains<sup>2</sup> carried single copy of IS6110 and 2-3% of the strains carry no IS6110 copy. Hence it becomes imperative to use an alternate

probe, like the direct-repeat (DR) probe, which is a DR element from *M. bovis* BCG to overcome the limitations of IS6110. We have used the DR probe to fingerprint isolates obtained from patients pretreatment and after a relapse to determine the frequency of exogenous reinfection versus the rate of endogenous reactivation in Madras in southern India.

### Comparison of pretreatment and post-treatment isolates in an urban clinical and laboratory setting

The pretreatment and relapse isolates of *M. tuberculosis* used in the study were obtained from patients with pulmonary tuberculosis in controlled clinical trials conducted at Tuberculosis Research Centre. The isolates originated from 52 patients who had undergone short-course Chemotherapy for 6 or 8 months. The initial isolate was obtained before starting treatment and the subsequent isolate was obtained after stopping treatment. Patients with quiescent disease were followed as long as 60 months by monthly sputum examination for upto 24 months from the start of treatment and at 3-month intervals thereafter in order to determine the stability of bacteriological quiescence and the relapse rates. A patient was classified as having had the bacteriological relapse (true relapse) if two or more positive cultures were obtained during a 6-month period.

Coded samples of 44 pairs of clinical isolates were analysed by RFLP with DR. On analysis 30 different patterns were observed and the number of bands ranged from two to seven. On the basis of the number and molecular sizes of the bands similar RFLP patterns were grouped. Our laboratory based study showed that the patterns of 69% of the isolates from patients with relapses matched those with pretreatment isolates, indicating that the rate of relapse caused by reactivation exceeds the rate of relapse caused by reinfection<sup>3</sup>.



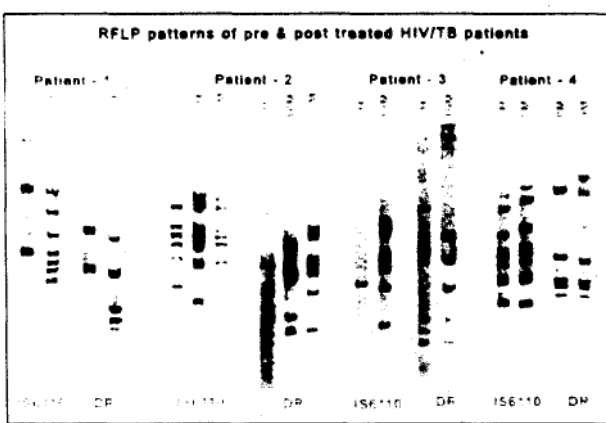
**Fig. 1 :** The DR RFLP patterns of the pre and post treatment isolates of patients  
lanes 1&2 represents pre & post treatment isolate of a patient and lanes 3&4 pre and post treatment isolate of the second patient and so on

Similar studies<sup>4</sup> were undertaken in a rural area of high prevalence in South India. The study site was Tiruvllur District, where the world's biggest *Mycobacterium bovis* BCG trial was

conducted<sup>5</sup>. The total population of the study area is 580,000. The incidence of smear positive TB is 76 per 100,000 in the population<sup>6</sup>. The study subjects were all TB patients undergoing treatment according to the Revised National Tuberculosis Control Programme guidelines. Of 437 culture positive patients 378 were available for RFLP analysis. Combined RFLP analysis with IS6110 & DR probes identified 236 (62%) patients with distinct patterns, and 142 (38%) patients were in one of the 35 clusters. More than 2 patients with identical RFLP patterns by IS6110 and DR probe were considered to be in a cluster. Totally there were 35 clusters in our first year study cluster denotes exogenous reinfection or ongoing transmission. The risk factors which were associated with clustering or ongoing transmission (though not statistically significant) are given below. Clustering was higher among patient above 45 years. House to house survey carried out in 10% of the population identified more patients in clusters. Furthermore, a majority of the patients who had a relapse and were in a cluster harbored an IS6110 single-copy strain. The risk factors not associated with clustering are multi-drug resistance, alcoholism, literacy and family size.

The findings of this study suggest that the majority of the TB cases in south India occur predominantly due to reactivation. With the large scale introduction of directly observed treatment - short course (DOTS) in 1998 the transmission of infection is likely to be reduced due to the higher cure and lower relapse rates. However the reactivation of latent infection will continue to perpetuate new cases for years to come. The DOTS implementation should be sustained and supplemented by intensive and early case detection of TB, infection control measures to prevent TB transmission at outpatient and inpatient health facilities, and community education.

The scenario has been different in HIV/TB. We have been following up the HIV/TB patients after treatment at Tuberculosis Research Centre. The positive cultures before treatment and after relapse have been analysed by RFLP using more than 2 probes. In contrast to non HIV/TB patients preliminary results indicate that the recurrent tuberculosis is more due to exogenous reinfection among HIV/TB. The study is in progress.



**Fig. 2 :** RFLP patterns of pre & post treated HIV/TB patients

Since exogenous reinfection has been that DOTS should be intensified. The other measure which could control TB in HIV patients is TB chemoprophylaxis of HIV positives and initiation of ART (anti retroviral therapy). The contribution of reinfection to the epidemiology and

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pathogenesis of tuberculosis has important implications for tuberculosis control in India and other countries with a high burden of HIV and tuberculosis and deserves further study.

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