ABSTRACT

Tuberculosis (TB) is the leading killer of adults in the world today among infectious diseases, and poses a serious challenge to international public health work. Thus there is a great concern about the world-wide magnitude of the new TB epidemic. The bacilli which causes TB, *Mycobacterium tuberculosis* affects about a third of the world's population. Western countries have enjoyed a long decrease in TB cases due to the control of population and crowding. However, TB is making a come back especially with the advent of Acquired Immuno Deficiency Syndrome (AIDS) epidemic.

The problem faced for effective control of TB in India is multitudinous. The diagnosis which is based mainly on chest x-ray and sometimes symptoms alone are common, and the quality and the supply of drugs is erratic. Infectious cases are frequently missed, while other people are mistakenly diagnosed with TB and inappropriately treated. As a result, at least two thirds of TB patients drop out of treatment early, often becoming chronic sufferers and sources of infection to others. These chronic cases may become incubators for the deadly drug-resistant bacterium.

Thus, it is apparent that timely diagnosis of mycobacterial infection is important both for clinical treatment of infected individuals and to identify persons who are at risk. Conventional diagnostic methods like sputum smear test, tuberculin test, chest x-ray and culture isolation have very poor sensitivity. Moreover, these tests cannot differentiate between the different species of mycobacteria. Hence, in the present study an attempt was made to diagnose mycobacterial infection in clinical specimens by Polymerase Chain Reaction
(PCR) based diagnostic method using the mycobacterium genus and *M. tuberculosis* specific DNA primers and probes.

Processing of blood samples for PCR analysis is difficult as compared to sputum samples. Haeme in blood is known to inhibit PCR. Hence, in the present study, a simple and rapid lysis protocol to get haeme free DNA lysates from blood samples for PCR analysis was developed. This protocol yielded DNA lysates from blood samples which worked well in PCR. The mycobacterium genus and *M. tuberculosis* specific primers and probes were used to diagnose mycobacterial infection from sputum and blood samples collected from tuberculosis, leprosy and AIDS patients. The primers and the probes were highly specific and sensitive which is unmatched by any conventional diagnostic method. This result suggests that PCR based diagnosis could be used for rapid detection of mycobacteria in the clinical specimens. Hence, refinements of the PCR technique and reduction in the costs will allow more extensive application of PCR in the future and facilitate rapid diagnosis of mycobacterial infection in local tuberculosis diagnostic centres.

In the second part of the study a semi-quantitative approach to correlate the bacilli load to the quantity of the PCR product using densitometric analysis was attempted to monitor treatment response in pulmonary tuberculosis patients undergoing chemotherapy. Semiquantitative PCR (SQ-PCR) for *M. tuberculosis* was performed using primers and probes specific for *M. tuberculosis*. It was observed that the SQ-PCR was able to demonstrate the patients progressively becoming negative as the treatment progresses. Moreover, no detectable signal was obtained from control samples (healthy individuals) included in the study. There was also a good correlation between culture isolation and SQ-PCR in the bacilli count observed during the course of chemotherapy. Hence, these results suggest a promising role for SQ-PCR as a tool to monitor treatment response in
pulmonary tuberculosis patients undergoing treatment.

An attempt was also made to develop *M. avium* specific primers and probes for the early detection of *M. avium*, an opportunistic pathogen frequently encountered in immunocompromised patients. The desired size PCR product was obtained after the initial standardisation and the primers and the probe were found to be highly specific for *M. avium*. These primers and probe were subsequently used on clinical isolates of *M. avium* and blood samples from HIV infected persons to detect *M. avium*. Sequence polymorphism exhibited by the different serovars of *M. avium* clinical isolates were also studied using PCR-SSCP and automated sequencing of the PCR amplified products. The results of the automated sequencing, subsequent BLAST analysis and multiple sequence alignment of the sequences using GenAlign programme from Intelligenetics demonstrated that the serovars 4 and 9 were closely related whereas serovar 8 was found to be different. These results show that there is genetic variability among the different serovars of *M. avium* in this particular region even though all the serovars give same size PCR product.

In the last part of the study, the prevalence of non-tuberculous mycobacteria (NTM) in tuberculosis, leprosy patients and the antibiotic sensitivity pattern of these NTM to various conventional and nonconventional mycobacterial drugs were investigated. The *M. fortuitum-chelonae* complex was found to be a major NTM infecting both leprosy and tuberculosis patients. Moreover, all the isolated NTM were found to be multi-drug resistant. To understand the implications of drug resistance of these organisms in the pathogenesis and control of leprosy and tuberculosis, molecular mechanisms of resistance to these drugs and biochemical changes consequent to the development of resistance need to be investigated in greater detail.