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MOLECULAR DIAGNOSIS OF SPECIFIC STRAINS OF *MYCOBACTERIA* IN KANDY AND MATALE DISTRICTS OF SRI LANKA

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RADHAKRISHNAN BALASUBRAMANIAM VASANTHAKRISHNAN

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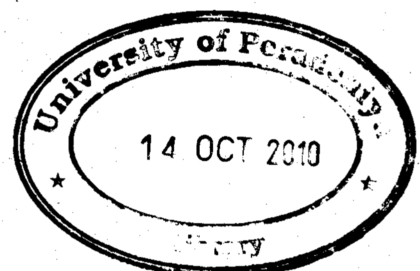

MOLECULAR DIAGNOSIS OF SPECIFIC STRAINS OF *MYCOBACTERIA* IN KANDY AND MATALE DISTRICTS OF SRI LANKA

R.K.B.S. Vasanthakrishnan
Department of Biochemistry
University of Peradeniya
Peradeniya
Sri Lanka

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* Complex (MtbC), is the leading cause of death, due to a single infectious agent worldwide. Since TB is highly infectious for humans, it is important that the disease be diagnosed as early as possible to stop the spread of the disease. In Sri Lanka laboratory diagnosis of tuberculosis is done by direct microscopic examination of stained sputum specimens for tubercle bacilli. However, specificity and sensitivity of this technique is not sufficient to identify the disease at early stage. Further it is impossible to identify MtbC sub species and Mycobacteria other than tuberculosis (MOTT) by this and culture techniques.

Examination by bacteriological culture provides conclusive diagnosis of TB. The culture technique of egg based solid media used in our county has the disadvantage of long delay (about 6 weeks) to obtain results, which is a significant delay in making a diagnosis. The polymerase chain reaction (PCR) based molecular diagnostic technique would overcome the deficiencies of the sputum direct microscopy and culture. It is possible to get rapid and accurate diagnosis of tuberculosis by using PCR.

This study focused on developing PCR based molecular diagnosis. The best method to extract DNA from three methods CTAB (Cetyltrimethyl ammonium bromide)/NaCl method, 2% SDS, 10% Triton X- 100 method and Tris-EDTA (TE)-boiling method of DNA extraction was identified. The methods were also evaluated with clinical samples.

Sputum specimens were collected from patients suspected of TB from the chest clinics of districts of Kandy and Matale and from the Microbiology laboratory at the University of Peradeniya. A total of 278 sputum samples were collected from 238 patients, and DNA was extracted using the CTAB/NaCl method. Fragment of extracted DNA was amplified by PCR with the primers PT-8 and PT-9 flanking a 541 bp sequence within the repetitive sequence IS *986* of MtbC. A PCR based method was developed to differentiate MtbC species from MOTT species. H37Rv strain DNA was used initially as a reference strain. Clinical samples that were positive by acid fast bacilli (AFB) staining, but negative by PCR for MtbC were tested to differentiate the MtbC strains from the MOTT species. PCR results and AFB staining results were compared with the clinical picture of the patients.

The CTAB/NaCl method was found to be the best method to obtain high purity DNA with a significant yield. Of the 278 clinical samples, 193 were positive for AFB, of which 124 were positive for MtbC by PCR and 69 were negative by PCR. Of the 85 AFB negative samples 8 were positive for MtbC by PCR while remaining 77 were negative.

Sixty nine clinical samples which were positive by smear and negative by PCR and 6 AFB negative and PCR negative samples from Matale and Kandy clinics were further analyzed using 16S rRNA, Rv0577, IS1561, Rv1510, Rv1970, Rv3877/8, and Rv3120 gene loci. Of the 75 samples, 20 were identified as species from MOTT. Out of the 20 patients one has died and 7 of them are chronically ill, in spite of getting TB treatment for 6 months. Remaining 12 patients are considered as cured. These results strongly suggest the importance of use of sensitive molecular diagnostic techniques for early detection and identification of the subspecies of *Mycobacteria* in Sri Lanka. Identification of the specific strains of MtbC and differentiating the MtbC and MOTT will improve treatment of patients with tuberculosis. Use molecular diagnosis will help the clinicians to ensure cure of individual patients and would prevent transmission of tuberculosis in Sri Lanka.