Tuberculosis Research Centre
Chennai

Annual Report

April 2003 – March 2004
## Contents

Preface \hspace{1cm} v  
Committees \hspace{1cm} vii  
Distinguished Visitors \hspace{1cm} xiv  
Guest Lectures \hspace{1cm} xvi  
Abbreviations \hspace{1cm} xvii  

### Research Activities

<table>
<thead>
<tr>
<th>Field</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Research</td>
<td>1</td>
</tr>
<tr>
<td>Sociological Research</td>
<td>4</td>
</tr>
<tr>
<td>Epidemiological Research</td>
<td>10</td>
</tr>
<tr>
<td>Operational Research</td>
<td>14</td>
</tr>
<tr>
<td>Applied Research</td>
<td>19</td>
</tr>
<tr>
<td>Basic Research</td>
<td>28</td>
</tr>
<tr>
<td>Statistical Research</td>
<td>48</td>
</tr>
<tr>
<td>Information Services</td>
<td>52</td>
</tr>
</tbody>
</table>

### Appendices

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Publications</td>
<td>54</td>
</tr>
<tr>
<td>Awards</td>
<td>59</td>
</tr>
<tr>
<td>Advocacy</td>
<td>59</td>
</tr>
<tr>
<td>Capacity Building</td>
<td>60</td>
</tr>
<tr>
<td>Conferences</td>
<td>61</td>
</tr>
<tr>
<td>Special Assignments</td>
<td>61</td>
</tr>
<tr>
<td>Ph.D. Scholars - Students</td>
<td>63</td>
</tr>
<tr>
<td>Ph.D. Scholars - Staff</td>
<td>65</td>
</tr>
</tbody>
</table>
The year under review was an eventful one with significant advances being made in the various research activities of TRC and the establishment of new facilities. During the year, construction of the long-awaited patient care and clinical research facility was inaugurated and is rapidly progressing. A notable landmark was achieved with the establishment of an International Centre of Excellence in Research (ICER) in collaboration with the National Institutes of Health, USA. This singular honour is the fruit of many years of very high quality work carried out by TRC in the fields of clinical and epidemiological research. The establishment of the ICER, apart from enhancing the reputation of TRC, will facilitate upgradation of facilities and open up opportunities for collaborative research and training in priority areas.

In the area of clinical trials, we made steady progress in recruiting subjects to two very important randomised clinical trials in HIV-infected persons. The first is a study of the efficacy of the RNTCP recommended 6-month regimen for treatment of pulmonary tuberculosis in HIV-seropositive patients as compared to an extended 9-month regimen. The second is a chemoprophylaxis study of the efficacy of two regimens for the prevention of active tuberculosis in HIV-seropositive subjects. Both these are crucial studies, which will help to answer very important questions for the nation’s health policies. We should have broad indicators about the efficacy of these regimens by next year.

The results of the intermittent ofloxacin study, in which the efficacy of a 4-month thrice-weekly ofloxacin-containing regimen was compared to a control thrice-weekly 6-month regimen for the treatment of patients with sputum-positive pulmonary tuberculosis, belied our expectations. The relapse rate in the test regimen cohort was unacceptably high and the study was closed prematurely in keeping with our reputation of maintaining high ethical standards. We are now embarking upon another randomized clinical trial with the newer generation of fluoroquinolones, gatifloxacin and moxifloxacin, which we are optimistic, will result in an effective 4-month thrice-weekly regimen in the near future.

The Model DOTS Project has entered its fifth year with many targets being reached earlier than planned. Following the baseline surveys of tuberculosis disease and infection in a community in Tiruvallur district, the first re-survey of tuberculosis disease has been completed. The results are being analysed. We now also have information for a five-year period on programme performance (case detection, sputum conversion and treatment outcome) of the RNTCP in one tuberculosis unit of Tiruvallur district, and the results are presented in this report. Many operational research questions are also being addressed in this field laboratory.

Good quality training is essential for successful implementation of the programme. TRC has been identified as a nodal centre for training in RNTCP by Central TB Division (CTD). TRC, though basically a research centre, accepted the challenge and started the training programme since 1999. All levels of health personnel are being trained at national and
international levels. During the period (April 2003-March 2004), we trained 48 medical officers in five batches, 28 medical officers in two batches, 83 senior treatment supervisors in five batches, 39 senior tuberculosis laboratory supervisors in six batches, 14 laboratory technicians in three batches and 49 field workers in four batches. A sensitisation programme on RNTCP was organised for Deputy Directors of Health Services (DDHS) and 21 officers participated in it. We also trained 14 programme managers—from DPR Korea (7), Myanmar (4), Bangladesh (2) and East Timor (1) – and one microbiologist from Bangladesh. Scientists from TRC were also invited as facilitators for training at national level to other states and international training programmes (SEARO).

Research in basic science progressed substantially in the reporting year. The regulatory role of vitamin D receptor gene variants, on vitamin D₃ modulated immune functions in pulmonary tuberculosis, suggested that normal subjects with BB, tt and FF genotypes may have down regulated immune functions and such individuals may be susceptible to tuberculosis. The ongoing project on construction of rBCG based HIV-1 epitope delivery system was completed. Cpn10-PND chimeric antigen was constructed and expressed in BCG. The role of Pkn1 protein, which plays a key role in cell growth and development, was further studied to explain the slow growth of *M. tuberculosis* and its ability to persist in the dormant state. The search for a sensitive and specific diagnostic test for early diagnosis of tuberculosis is still on. Studies on cell proliferation and apoptosis induced by mycobacterial antigens in tuberculous pleuritis are in progress.

The recently formed Division of HIV/AIDS has initiated a number of laboratory studies aimed at understanding the immune responses in the HIV/TB co-infected patients. TRC is planning a randomised clinical trial with antiretroviral agents. In this context the laboratory has also taken up pharmacokinetic studies to identify drug interactions and assessment of the quality of antiretroviral agents. Using molecular typing methods to compare the genotypes of pretreatment and relapse isolates of *M. tuberculosis* from tuberculosis patients, we observed that exogenous re-infection was more common among patients with HIV/TB and endogenous reactivation commoner in HIV-seronegative patients with tuberculosis.

It is heartening to inform you that as in the past, in the reporting year also, TRC has shared its expertise with local, national, regional and international agencies, institutions and individuals in various ways. Many of the scientists and technical staff have served as collaborators, consultants, coordinators, discussants, experts, partners and temporary advisors in tuberculosis control and research.

Several young students who have recently joined (and the number is increasing year after year) and registered with University of Madras to work for their Ph.D. at TRC will ensure that the laboratory infrastructure being developed will be used optimally.

In summary, while clinical trials and epidemiology continue to be our forte, we are also making special efforts to strengthen our presence in basic science research. We are hopeful that these efforts will yield results in the near future. I thank all the staff of TRC for their outstanding efforts in continuing to keep the status of TRC “high” in the TB research world. I would like to acknowledge the timely funding received by TRC from USAID, WHO, NIAID and UCLA that supported research projects relevant to the health needs of our country. I thank Prof. N.K. Ganguly, our Director General, and ICMR for continued encouragement and unstinted support. We realise that the DG, by posing challenge after challenge to TRC, is striving to make us leaders in TB research, control and training. I take this occasion to thank the Ministry of Health, Government of India, in general and the CTD in particular for according us the status of one of the “most favoured partners” in all aspects of TB control and research. Finally I thank the Corporation of Chennai and the Government of Tamil Nadu for their continued support and providing valuable feedback on our efforts in TB control, research and training.

Dr. P.R. Narayanan
Director
Scientific Advisory Committee

Chairperson
Dr. C.M. Gupta
Director
Central Drug Research Institute
Lucknow

Members
Prof. K.V. Thiruvengadam
Former Professor of Medicine
Madras Medical College
Chennai

Dr. S.P. Tripathy
Former Director General, ICMR
Pune

Dr. S. Radhakrishna
Former Director, IRMS
Hyderabad

Prof. V.I. Mathan
Consultant to ICMR
National Institute of Epidemiology
Chennai

Dr. Lalji Singh
Director
Centre for Cellular and Molecular Biology (CCMB)
Hyderabad

Dr. L.S. Chauhan
Deputy Director General (TB)
Directorate General of Health Services
New Delhi

Dr. M.K. Bhan
Professor of Paediatrics
All India Institute of Medical Sciences
New Delhi

Prof. V. Nagaraja
Professor
Indian Institute of Science
Bangalore

Prof. Anil K. Tyagi
Professor of Biochemistry
University of Delhi
(South Campus)
New Delhi

Dr. Dipali Mukherji
Chief, Division of ECD
Indian Council of Medical Research
New Delhi

Special Invitees
Dr. P. Vijayalakshmi
Director of Medical Education
Government of Tamil Nadu
Chennai

Dr. P. Krishnamurthy
Director of Public Health & Preventive Medicine
Chennai

Dr. J.R. Vijayalakshmi
Director of Medical & Rural Health Services
Chennai

Dr. M. Perumal
State TB Officer &
Joint Director of Medical & Rural Health Services (TM)
Directorate of Medical & Rural Health Services
Chennai
Prof. A.S. Natarajan  
Director  
Institute of Thoracic Medicine  
Chennai  

Dr. Prahlad Kumar  
Director  
National Tuberculosis Institute  
Bangalore  

Dr. Seyed E. Hasnain  
(DG’s Nominee)  
Director  
Centre for DNA Fingerprinting and Diagnostics  
Hyderabad  

Prof. S.C. Sehgal  
Chief, International Health Division  
Indian Council of Medical Research  
New Delhi  

Dr. M.D. Gupte  
Director  
National Institute of Epidemiology  
Chennai  

Dr. P.K. Das  
Director  
Vector Control Research Centre  
Pondicherry  

Dr. V.M. Katoch  
Director  
Central Jalma Institute for Leprosy  
Agra  

Dr. P.R. Narayanan (Member-Secretary)  
Tuberculosis Research Centre  
Chennai
Ethics Committee

Chairperson

Dr. Jacob Abraham
Consultant Neurosurgeon
Chennai

Members

Dr. H. Srinivasan
Honorary Editor
Indian Journal of Leprosy
Chennai

Mr. Mohan Alagappan
Industrialist
Chennai

Dr. Usha Sriram
Clinical Endocrinologist
E.V. Kalyani Medical Centre
Chennai

Dr. Vijayalakshmi Thanasekaran
Consultant Pulmonologist
Sri Ramachandra Medical College
& Research Institute
Chennai

Dr. Uma Krishnaswamy
Consultant General Surgeon
Chennai

Dr. V.R. Muraleedharan
Professor of Economics
Indian Institute of Technology
Chennai

Ms. Shyamala Nataraj
Programme Director
South India AIDS Action Programme (NGO)
Chennai

Dr. V. Vijayasekaran
Professor and Head
Department of Pharmacology
Vinayaka Mission Medical College
Salem

Dr. Rema Mathew (Member-Secretary)
Deputy Director
Tuberculosis Research Centre
Chennai
Animal Ethics Committee

1. Mrs. Prema Veeraraghavan, No.172 (Old No. 94), 13, Kala Flats, Habibullah Road, T. Nagar, Chennai – 600 017

2. Dr. P. Krishnamoorthy, No.40, Third Street, V.R. Puram, Saligramam, Chennai – 600 093

3. Dr. Rama Rajaram, Scientist E, CLRI, Adyar, Chennai – 600 020
Abstract Committee
Dr. Rani Balasubramanian (Chairperson)
Dr. Vanaja Kumar
Mrs. Fathima Rahman
Dr. T. Santha Devi
Dr. Sujatha Narayanan
Dr. P. Venkatesan (Member-Secretary)

Animal House Committee
Dr. Rajeswari Ramachandran (Chairperson)
Dr. K.V. Kuppu Rao
Dr. Sulochana Das
Dr. K. Jayasankar
Dr. P. Kannan (Member-Secretary)

Annual Report Committee
Dr. M.S. Jawahar (Chairperson)
Mrs. Fathima Rahman
Dr. Geetha Ramachandran (Member-Secretary)
Dr. N. Selva Kumar
Dr. P. Paul Kumaran

Anti-Women Harassment Committee
Dr. Soumya Swaminathan (Chairperson)
Mr. M. Mani
Dr. Geetharamani Shanmugham
Mr. V. Adikesavan (Member-Secretary)

Bio-safety Committee
Dr. Vanaja Kumar (Chairperson)
Dr. Sulochana Das
Dr. V.D. Ramanathan
Dr. M. Kannapiran (Member-Secretary)

Building Committee
Dr. C.N. Paramasivan (Chairperson)
Dr. M.S. Jawahar
Dr. V.D. Ramanathan
Dr. P. Venkatesan
Mr. M. Subramanian (Member-Secretary)

Canteen Committee
Mr. R. Subramani (Chairperson)
Mr. V. Venkatesh Prasad
Mr. R. Balakrishnan
Ms. Nazeema Beevi
Ms. Arumaikannu Anbalagan
Ms. Beena Elizabeth Thomas
Ms. M. Vijayalakshmi
Dr. K. Jayasankar
Mr. K. Krishnan
Mr. E. Raman
Mr. P.B. Madhavan
Mr. N.C. Sridharan (Member-Secretary)

Celebration Committee
Dr. V. Kumaraswami (Chairperson)
Mr. M. Subramanian
Mr. E. Money
Mr. D. Shanmugam
Mr. P.B. Madhavan
Mr. S.N. Sankaralingam
Mr. V. Adikesavan (Member-Secretary)
Committee of Heads of Departments

Dr. P.R. Narayanan (Chairperson)
Dr. C.N. Paramasivan
Dr. Aleyamma Thomas
Dr. V. Kumaraswami
Dr. Rajeswari Ramachandran
Dr. V.D. Ramanathan
Dr. Soumya Swaminathan
Dr. C. Kolappan
Dr. M. Kannapiran
Mr. P.G. Gopi
Mr. R. Subramani
Dr. K. Jayasankar
Mr. M. Subramanian
Mr. V. Adikesavan
Mr. N.C. Sridharan
Dr. P. Venkatesan (Member-Secretary)

Mr. D. Shanmugam
Mr. R.S. Somasundaram
Mr. S.N. Sankaralingam (Member-Secretary)

Infection Control Committee

Dr. V. Kumaraswami (Chairperson)
Dr. Soumya Swaminathan
Mr. M. Subramanian
Mr. S. Ramanujam
Ms. S. Padma
Mr. D. Shanmugam
Dr. Ranjani Ramachandran (Member-Secretary)

Internal Ethics Committee

Dr. Rema Mathew (Chairperson)
Dr. K. Sadacharam
Dr. Rani Balasubramanian
Dr. Pauline Joseph (Member-Secretary)

Library Committee

Dr. V. Kumaraswami (Chairperson)
Dr. V.D. Ramanathan
Dr. Sujatha Narayanan
Dr. Pauline Joseph
Mr. R. Rathinasabapati (Member-Secretary)

Purchase Committee

Dr. N. Selva Kumar (Chairperson)
Dr. Aleyamma Thomas
Dr. M. Kannapiran (Member-Secretary)

Research Committee

Dr. N. Selvakumar (Chairperson)
Dr. V. Chandrasekaran
Mrs. Fathima Rahman
Dr. Geetha Ramachandran
Dr. A.K. Hemanth Kumar
Dr. G. Kubendiran
Dr. Luke Elizabeth Hanna
Dr. Ranjani Ramachandran
Dr. P. Selvaraj
Mr. R. Selvaraj
Dr. Sujatha Narayanan
Dr. Sulochana Das
Dr. Vanaja Kumar
Dr. Alamelu Raja (Member-Secretary)

Sr. Technical Officers and Technical Officers Committee

Dr. K. Jayasankar (Chairperson)
Mrs. Jaya Gopinath
Mr. S.N. Sankaralingam
Mr. K.J. Illampurnam
Mrs. Lalitha Victor
Mrs. Nalini Sundar Mohan
Mrs. S. Sudhamathi
Mr. E. Money
Mr. S. Ramanujam (Member-Secretary)

Transport Committee

Dr. M.S. Jawahar (Chairperson)
Mr. E. Money
Ms. Jayalakshmi Vadivel
Mr. V. Adikesavan (Member-Secretary)

Vigilance Committee

Dr. V.D. Ramanathan (Chairperson)
Dr. P. Selvaraj
Ms. Sudha Ganapathy
Mr. T.M. Kasinathan (Member-Secretary)
<table>
<thead>
<tr>
<th>Name</th>
<th>Position/Title</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Martien Borgdoff</td>
<td>Epidemiologist</td>
<td>The Netherlands</td>
</tr>
<tr>
<td></td>
<td>KNCV</td>
<td></td>
</tr>
<tr>
<td>Dr. Christopher Dye</td>
<td>Division of Communicable Diseases</td>
<td>Switzerland</td>
</tr>
<tr>
<td></td>
<td>Control and Prevention</td>
<td></td>
</tr>
<tr>
<td></td>
<td>World Health Organization</td>
<td></td>
</tr>
<tr>
<td>Dr. Fabio Luelmo</td>
<td>TB Consultant</td>
<td>Switzerland</td>
</tr>
<tr>
<td></td>
<td>Division of Communicable Diseases</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control and Prevention</td>
<td></td>
</tr>
<tr>
<td></td>
<td>World Health Organization</td>
<td></td>
</tr>
<tr>
<td>Dr. Brian Williams</td>
<td>Division of Communicable Diseases</td>
<td>Switzerland</td>
</tr>
<tr>
<td></td>
<td>Control and Prevention</td>
<td></td>
</tr>
<tr>
<td></td>
<td>World Health Organization</td>
<td></td>
</tr>
<tr>
<td>Dr. Eric Chamot</td>
<td>Assistant Professor</td>
<td>USA</td>
</tr>
<tr>
<td></td>
<td>University of Alabama</td>
<td></td>
</tr>
<tr>
<td></td>
<td>at Birmingham</td>
<td></td>
</tr>
<tr>
<td>Mr. Jack C. Chow</td>
<td>Ambassador</td>
<td>USA</td>
</tr>
<tr>
<td></td>
<td>Deputy Assistant Secretary</td>
<td></td>
</tr>
<tr>
<td></td>
<td>for Health and Science</td>
<td></td>
</tr>
<tr>
<td></td>
<td>U.S. Department of State</td>
<td></td>
</tr>
<tr>
<td>Dr. Jose A Caminero Luna</td>
<td>Consultant</td>
<td>Spain</td>
</tr>
<tr>
<td></td>
<td>International Union Against Tuberculosis and Lung Diseases</td>
<td></td>
</tr>
<tr>
<td>Mr. Jonathan Folkers</td>
<td>Computer Specialist</td>
<td>USA</td>
</tr>
<tr>
<td></td>
<td>National Institutes of Health</td>
<td></td>
</tr>
<tr>
<td>Ms. Sibylle Kristensen</td>
<td>Program Director</td>
<td>USA</td>
</tr>
<tr>
<td></td>
<td>University of Alabama</td>
<td></td>
</tr>
<tr>
<td></td>
<td>at Birmingham</td>
<td></td>
</tr>
<tr>
<td>Dr. Marion E Broom</td>
<td>Professor/ Associate Dean</td>
<td>USA</td>
</tr>
<tr>
<td></td>
<td>University of Alabama</td>
<td></td>
</tr>
<tr>
<td></td>
<td>at Birmingham</td>
<td></td>
</tr>
<tr>
<td>Dr. Polly Sager</td>
<td>International Research Support &amp; Training</td>
<td>USA</td>
</tr>
<tr>
<td></td>
<td>National Institutes of Health</td>
<td></td>
</tr>
<tr>
<td>Dr. Anette Haller</td>
<td>Programme Advisor</td>
<td>Thailand</td>
</tr>
<tr>
<td></td>
<td>Regional Bureau of Asia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UN World Food Programme</td>
<td></td>
</tr>
<tr>
<td>Ms. Caroling Legros</td>
<td>World Food Programme</td>
<td>India</td>
</tr>
<tr>
<td>Name</td>
<td>Position and Affiliation</td>
<td>Country</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-------------------------------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Dr. Charles Wells</td>
<td>Chief International Activity Centers for Disease Control (CDC) and Prevention</td>
<td>USA</td>
</tr>
<tr>
<td>Dr. Marguerite Pappanaiou</td>
<td>Centers for Disease Control (CDC) and Prevention</td>
<td>USA</td>
</tr>
<tr>
<td>Dr. Michael Boom</td>
<td>Centers for Disease Control (CDC) and Prevention</td>
<td>USA</td>
</tr>
<tr>
<td>Dr. Rodrigo L.C. Romulo</td>
<td>Chairman Philippine TB initiative for the private sector</td>
<td>Philippines</td>
</tr>
<tr>
<td>Dr. Richard M. Krause</td>
<td>Senior Investigator NIAID National Institutes of Health</td>
<td>USA</td>
</tr>
<tr>
<td>Dr. Kathy DeRiemer</td>
<td>Division of Infectious Diseases and Geographic Medicine Stanford University</td>
<td>USA</td>
</tr>
<tr>
<td>Dr. Cristina Coutierret</td>
<td>Institut Pasteur at Paris</td>
<td>France</td>
</tr>
<tr>
<td>Dr. Philip Supply</td>
<td>Institut Pasteur at Paris</td>
<td>France</td>
</tr>
<tr>
<td>Dr. Bess Miller</td>
<td>Associate Director for TB/HIV Prevention and Care Centers for Disease Control (CDC) and Prevention</td>
<td>USA</td>
</tr>
<tr>
<td>Dr. Naomi Bock</td>
<td>Global AIDS program Centers for Disease Control (CDC) and Prevention</td>
<td>USA</td>
</tr>
<tr>
<td>Dr. Jamie Da Costa Sarmento</td>
<td>Director NTP of East Timor</td>
<td>East Timor</td>
</tr>
<tr>
<td>Dr. Harold Jaffe</td>
<td>Centers for Disease Control (CDC) and Prevention</td>
<td>USA</td>
</tr>
<tr>
<td>Dr. Susan M. Bacheller</td>
<td>Senior Infectious Disease Advisor Office of the HIV/AIDS Bureau for Global Health, USAID</td>
<td>USA</td>
</tr>
<tr>
<td>Dr. Amy Bloom</td>
<td>Senior Technical Advisor USAID</td>
<td>USA</td>
</tr>
<tr>
<td>Dr. Irene Kolk</td>
<td>Chief, Infectious Diseases Division, Bureau for Global Health, USAID</td>
<td>USA</td>
</tr>
</tbody>
</table>
Guest lectures during the year

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Christine Wanke</td>
<td>Tufts University School of Medicine, USA</td>
<td>HIV, nutrition and metabolism</td>
</tr>
<tr>
<td>Dr. Jose A Caminero</td>
<td>International Union against Tuberculosis and Lung Diseases, France</td>
<td>Standardised vs individualised treatment for MDR-TB</td>
</tr>
<tr>
<td>Dr. Vaidehi</td>
<td>Sundaram Medical Foundation, Chennai</td>
<td>Waste management</td>
</tr>
<tr>
<td>Ms. Sumathi Sundaram</td>
<td>London School of Tropical Medicine and Hygiene, UK</td>
<td>Tracking financial flows of health research and development in India</td>
</tr>
<tr>
<td>Dr. Kathy DeRiimer</td>
<td>Stanford University School of Medicine, USA</td>
<td>Comparative genomics of <em>M. tuberculosis</em></td>
</tr>
<tr>
<td>Dr. Nalini Sathiakumar</td>
<td>University of Alabama at Birmingham, USA</td>
<td>Epidemiological research tools in control of infectious diseases</td>
</tr>
<tr>
<td>Dr. Mathur S. Kannan</td>
<td>Professor, Dept. of Veterinary Pathobiology, College of Veterinary Medicine, USA</td>
<td>Calcium regulation in airway cell: signaling mechanism</td>
</tr>
<tr>
<td>Dr. S. Jambunathan</td>
<td>Physician, St. Johns Hospital, Asst. Professor of Medicine, Wayne State University, USA</td>
<td>Community acquired pneumonias</td>
</tr>
<tr>
<td>Dr. Richard M. Krause</td>
<td>Senior Investigator, National Institutes of Health, USA</td>
<td>Reflections on the discovery of group A streptococci as a cause of rheumatic fever</td>
</tr>
<tr>
<td>Dr. Kenneth Mayer</td>
<td>Professor of Medicine, Brown University School of Medicine, USA</td>
<td>HIV prevention research</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>AFB</td>
<td>Acid Fast Bacilli</td>
<td></td>
</tr>
<tr>
<td>AIDS</td>
<td>Acquired Immunodeficiency Syndrome</td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>Auramine Phenol</td>
<td></td>
</tr>
<tr>
<td>ATT</td>
<td>Anti-TB treatment</td>
<td></td>
</tr>
<tr>
<td>AUDIT</td>
<td>Alcohol Use Disorders Identification Test</td>
<td></td>
</tr>
<tr>
<td>BCG</td>
<td>Bacillus Calmette Guerin</td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>Cystic Fibrosis</td>
<td></td>
</tr>
<tr>
<td>CFA</td>
<td>Culture Filtrate Antigen</td>
<td></td>
</tr>
<tr>
<td>CFU</td>
<td>Colony Forming Unit</td>
<td></td>
</tr>
<tr>
<td>CPC</td>
<td>Cetyl Pyridinium Chloride</td>
<td></td>
</tr>
<tr>
<td>CTD</td>
<td>Central TB Division</td>
<td></td>
</tr>
<tr>
<td>DOTS</td>
<td>Directly Observed Treatment Short Course</td>
<td></td>
</tr>
<tr>
<td>EMB</td>
<td>Ethambutol</td>
<td></td>
</tr>
<tr>
<td>ELISpot</td>
<td>Enzyme Linked Immunosorbent Spot</td>
<td></td>
</tr>
<tr>
<td>FEV</td>
<td>Forced Expiratory Volume</td>
<td></td>
</tr>
<tr>
<td>FVC</td>
<td>Forced Vital Capacity</td>
<td></td>
</tr>
<tr>
<td>GAT</td>
<td>Gatifloxacin</td>
<td></td>
</tr>
<tr>
<td>HAART</td>
<td>Highly Active Antiretroviral Therapy</td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
<td></td>
</tr>
<tr>
<td>HLA</td>
<td>Human Leucocyte Antigen</td>
<td></td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
<td></td>
</tr>
<tr>
<td>IEC</td>
<td>Information, Education and Communication</td>
<td></td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
<td></td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
<td></td>
</tr>
<tr>
<td>INH</td>
<td>Isoniazid</td>
<td></td>
</tr>
<tr>
<td>IPTG</td>
<td>Isopropyl thiogalactopyranoside</td>
<td></td>
</tr>
<tr>
<td>IU</td>
<td>International Unit</td>
<td></td>
</tr>
<tr>
<td>LQAS</td>
<td>Lot Quality Assurance Sampling</td>
<td></td>
</tr>
<tr>
<td>LRP</td>
<td>Luciferase Reporter Phage</td>
<td></td>
</tr>
<tr>
<td>LT</td>
<td>Laboratory Technician</td>
<td></td>
</tr>
<tr>
<td>MABA</td>
<td>Microplate Alamar Blue Assay</td>
<td></td>
</tr>
<tr>
<td>MAC</td>
<td>M. avium Complex</td>
<td></td>
</tr>
<tr>
<td>MBL</td>
<td>Mannose Binding Lectin</td>
<td></td>
</tr>
<tr>
<td>MDR-TB</td>
<td>Multidrug Resistant Tuberculosis</td>
<td></td>
</tr>
<tr>
<td>MGIT</td>
<td>Mycobacterial Growth Indicator Tube</td>
<td></td>
</tr>
<tr>
<td>MIP</td>
<td>Macrophage Inflammatory Protein</td>
<td></td>
</tr>
<tr>
<td>MX</td>
<td>Moxifloxacin</td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium Chloride</td>
<td></td>
</tr>
<tr>
<td>NHS</td>
<td>Normal Healthy Subjects</td>
<td></td>
</tr>
<tr>
<td>NPV</td>
<td>Negative Predictive Value</td>
<td></td>
</tr>
<tr>
<td>NTM</td>
<td>Non-Tuberculous Mycobacteria</td>
<td></td>
</tr>
<tr>
<td>OFX</td>
<td>Ofloxacin</td>
<td></td>
</tr>
<tr>
<td>PBMC</td>
<td>Peripheral Blood Mononuclear Cells</td>
<td></td>
</tr>
<tr>
<td>PF</td>
<td>Pleural Fluid</td>
<td></td>
</tr>
<tr>
<td>PFGE</td>
<td>Pulsed Field Gel Electrophoresis</td>
<td></td>
</tr>
<tr>
<td>PFMC</td>
<td>Pleural Fluid Mononuclear Cells</td>
<td></td>
</tr>
<tr>
<td>PGRS</td>
<td>Polymorphic GC Repeat Sequence</td>
<td></td>
</tr>
<tr>
<td>PHA</td>
<td>Phytohaemagglutinin</td>
<td></td>
</tr>
<tr>
<td>PMN</td>
<td>Polymorphonuclear</td>
<td></td>
</tr>
<tr>
<td>PND</td>
<td>Principal Neutralizing Determinant</td>
<td></td>
</tr>
<tr>
<td>PPV</td>
<td>Positive Predictive Value</td>
<td></td>
</tr>
<tr>
<td>PZA</td>
<td>Pyrazinamide</td>
<td></td>
</tr>
<tr>
<td>QRDR</td>
<td>Quinolone Resistance Determining Region</td>
<td></td>
</tr>
<tr>
<td>RFLP</td>
<td>Restriction Fragment Length Polymorphism</td>
<td></td>
</tr>
<tr>
<td>RLU</td>
<td>Relative Light Unit</td>
<td></td>
</tr>
<tr>
<td>RMP</td>
<td>Rifampicin</td>
<td></td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>RNTCP</td>
<td>Revised National Tuberculosis Control Programme</td>
<td></td>
</tr>
<tr>
<td>SCC</td>
<td>Short Course Chemotherapy</td>
<td></td>
</tr>
<tr>
<td>SM</td>
<td>Streptomycin</td>
<td></td>
</tr>
<tr>
<td>SMR</td>
<td>Standardised Mortality Ratio</td>
<td></td>
</tr>
<tr>
<td>STD</td>
<td>Sexually Transmitted Disease</td>
<td></td>
</tr>
<tr>
<td>STLS</td>
<td>Senior Tuberculosis Laboratory Supervisor</td>
<td></td>
</tr>
<tr>
<td>TB</td>
<td>Tuberculosis</td>
<td></td>
</tr>
<tr>
<td>TLC</td>
<td>Total Lymphocyte Count</td>
<td></td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like Receptor</td>
<td></td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
<td></td>
</tr>
<tr>
<td>TU</td>
<td>Tuberculosis Unit</td>
<td></td>
</tr>
<tr>
<td>VCTC</td>
<td>Voluntary Counselling and Testing Centre</td>
<td></td>
</tr>
<tr>
<td>VDR</td>
<td>Vitamin D Receptor</td>
<td></td>
</tr>
<tr>
<td>VHN</td>
<td>Village Health Nurse</td>
<td></td>
</tr>
<tr>
<td>ZN</td>
<td>Ziehl-Neelsen</td>
<td></td>
</tr>
</tbody>
</table>
Overview of the research activities of the chemotherapy division

Over the last five years Tuberculosis Research Centre has carried out a number of clinical trials with special reference to the nation’s tuberculosis control programme. Two such important studies were conducted with the primary aim of shortening the duration of treatment for sputum-positive pulmonary tuberculosis. The first study in which ofloxacin was substituted for ethambutol in the standard 4-drug regimen has been hailed as a major breakthrough in tuberculosis treatment. The study showed that a 4-month daily regimen containing ofloxacin, rifampicin, isoniazid and pyrazinamide for the first three months is highly effective in treating patients with smear-positive pulmonary tuberculosis, thus proving for the first time that it is feasible to shorten tuberculosis treatment from six to four months (Annual Report 2001-2002). A follow-up study in which a 4-month ofloxacin containing intermittent (thrice-weekly) regimen was compared to the standard 6-month treatment was however disappointing. The relapse rate in the 4-month test regimen was unacceptably high and so the study was terminated mid-way (Annual Report 2002-2003). However, the Centre is now carrying out a similar study using either gatifloxacin or moxifloxacin instead of ofloxacin in an endeavour to find an effective 4-month thrice-weekly regimen that can be used by the RNTCP.

The Centre also carried out a clinical trial to study the efficacy of an 8-month daily regimen that used a non-rifampicin continuation phase (ethambutol plus isoniazid) in the treatment of smear-positive pulmonary tuberculosis patients. This regimen would be of particular use in situations where rifampicin cannot be used (as in concomitant antiretroviral therapy) and would hopefully obviate the need to give the rifampicin-containing continuation phase unsupervised. This study also investigated the role of extension of the intensive phase of treatment if the sputum is smear-positive at the end of two months (Annual Report 2002-2003).

Diabetes mellitus is a known risk factor for tuberculosis. The Centre has carried out a study to assess the efficacy of the RNTCP Category I regimen for treatment of new smear-positive pulmonary tuberculosis patients with concomitant Type II non-insulin dependent diabetes mellitus. The results are encouraging and have shown that the Category I regimen is adequate for the treatment of such patients (Annual Report 2002-2003).

Studies in progress
A study of the efficacy and tolerability of moxifloxacin and gatifloxacin containing regimens in the treatment of patients with sputum positive pulmonary tuberculosis
Moxifloxacin and gatifloxacin have been shown to have excellent activity (*in vitro* and in animal models of tuberculosis), a favourable pharmacokinetic profile (serum half-life of 10 – 12 hours) and safety profile.
A randomised controlled clinical trial has been started to assess the tolerability and efficacy of gatifloxacin and moxifloxacin containing regimens of four months duration in the treatment of new smear-positive pulmonary tuberculosis patients.

Regimen 1: 2GHRZ₃ / 2GHR₃. Gatifloxacin, isoniazid, rifampicin and pyrazinamide thrice-weekly for two months followed by gatifloxacin, isoniazid and rifampicin thrice-weekly for two months.

Regimen 2: 2MHRZ₃ / 2MHR₃. Moxifloxacin, isoniazid, rifampicin and pyrazinamide thrice-weekly for two months followed by moxifloxacin, isoniazid and rifampicin thrice-weekly for two months.

Regimen 3: 2EHRZ₃ / 4HR₃ as a control regimen. It is proposed to admit 300 patients to each limb. The study is being conducted in Chennai and Madurai.

Evaluation of chemotherapy regimens for tuberculosis in HIV-infected persons
This study was conducted to assess the efficacy of RNTCP regimens among HIV-infected persons with pulmonary tuberculosis or tuberculous lymphadenitis and to assess any additional benefit from an extended continuation phase (six month vs nine month regimen). Up to March 2004, 271 patients have been admitted to the study – 120 in Category I, 117 in Category I with extended continuation phase (trial regimen), 30 in Category II and 4 in Category III. Patients are being followed up to assess cure and relapse rates.

Preventive therapy for tuberculosis in HIV-infected persons
The aim of this study was to compare the efficacy of two regimens (isoniazid 300mg daily for three years versus ethambutol 800mg and isoniazid 300mg daily for six months) in reducing the incidence of tuberculosis and mortality among HIV-infected persons. Up to March 2004, 477 HIV-infected individuals have been admitted to the study. A five-year follow-up is planned to assess TB incidence and mortality rates in the two groups. The study is being conducted simultaneously in Chennai and Madurai. Intake was started in January 2001.

Long-term status of sputum-positive pulmonary tuberculosis patients successfully treated with short course chemotherapy (SCC)
A study has been initiated to get information on the long-term clinical, radiological and bacteriological
status of the patients successfully treated with SCC regimen. Hence a one-time assessment of patients at 10 – 15 years after completion of follow-up period is being undertaken.

Lung function impairment in patients treated for pulmonary tuberculosis and the role of inhaled steroids – a double blind randomised trial

The objectives of this study are:
a) To characterize the physiological and functional changes/abnormalities in lung function in patients treated for pulmonary tuberculosis; and

b) To study the effect of inhaled steroids in reducing or reversing the residual defect.

Two hundred and eighty-three patients have completed pulmonary function testing performed at third month of anti-TB treatment (ATT). Eighty-seven patients were found to have abnormalities and were allocated to inhaled steroid or placebo along with ATT for next three months. These patients are being followed-up with periodic pulmonary function tests up to 24 months. The follow-up phase of the study is likely to be completed by March 2005.

Nutritional assessment and supplementation in HIV-infected patients with and without tuberculosis

The main aims of the study are:
a) To document the occurrence of baseline macro- and micronutrient-deficiencies in HIV-infected individuals in south India and correlate that with their immune status; and

b) To test the efficacy of an intervention, in the form of a nutritional supplement and to quantitate changes in nutritional, biochemical and immunological parameters over a period of two years.

Two hundred and forty-two asymptomatic HIV-infected individuals and 87 HIV/TB patients have been included in this study. A high calorie, high protein supplement "Indiamix", supplied by the World Food Programme, New Delhi, is given to patients with the advice to consume 100gm per day. Patients will be followed up and investigations repeated at the end of one year to assess the response to this intervention.
Studies Completed

Factors influencing the care-seeking behaviour of chest symptomatics: A community-based study involving rural and urban population in Tamil Nadu, south India

Background and aim
The Revised National Tuberculosis Control Programme relies upon the passive method of screening chest symptomatics who seek care at health facilities. A community-based study was undertaken in two urban and two rural areas in southern India to identify the factors that influence the care-seeking behaviour of chest symptomatics.

Methods
Trained and experienced medical social workers interviewed each participant with the aid of a structured, precoded and pre-tested questionnaire.

Results
Of the 8818 urban and 6309 rural adults interviewed, 340 (3.9%) and 349 (5.5%), respectively, reported chest symptoms. The prevalence of chest symptoms among urban and rural symptomatics increased with age (p < 0.001); it was similar in men and women. Eighty percent of 310 urban residents and 63% of 339 rural people had sought care (p < 0.01), 93% within one month of the onset of symptoms. Overall, a greater percentage of symptomatics attended private health care facilities (urban 78%, rural 65%) than a governmental one (urban 55%, rural 60%); while 33% of urban and 21% of rural chest symptomatics opted for self-medication. Only 4% of rural symptomatics resorted to home remedies and alternate system of medicine such as homoeopathy, ayurveda or siddha, compared to 14% of urban chest symptomatics. Chest symptomatics commonly switched from one type of health care provider to another. Unaffordability was the commonest reason given for shifting from private to governmental facility, while the main reasons given for switching from a governmental to a private facility were dissatisfaction with the services and the location of the health facility (far from their residence).

The delay in care-seeking was significantly higher among symptomatics above 45 years of age (p = 0.02) and among illiterates (p = 0.05). Symptomatics who had previously taken treatment for tuberculosis waited longer to seek care than those without history of previous treatment (p = 0.02). Higher literacy was significantly associated with care seeking. The reasons why people did not seek medical advice are presented in Table 1. The main causes are lack of severity of symptoms (51%) and unaffordability (46%).

Conclusion
The finding that a significant proportion of illiterate participants, especially those in the rural areas, did not take action or delayed taking action underscores the importance of increasing awareness among the rural populations about the chest symptoms for TB and that information, education and communication (IEC) materials must be tailored to suit their special needs. We also observed a general participant dissatisfaction with the health
system, as evidenced by the fact that about half the patients shifted away from the facility they approached initially. It is clear that besides expanding the governmental health care network, especially in rural areas, they must be made more accessible, communicative and convenient. Our study highlights the need to involve the private medical sector in tuberculosis control and to make governmental health facilities more accessible and user friendly.

**Patient-preferred DOTS providers for tuberculosis treatment in HIV-infected individuals: A pilot study**

**Background**

Health facility-based DOT for all tuberculosis patients is not practical due to high case load and often requires patients to travel long distances every day. An alternative method is to involve community members in tuberculosis treatment delivery. This study summarises the role of community DOT providers in management of tuberculosis treatment in HIV-infected persons in a mainly rural population of Tamil Nadu.

**Aims**

a) To evaluate the feasibility of employing community DOT providers for tuberculosis treatment among HIV-infected persons;

b) To find out their influence on patient treatment compliance; and

c) To study patients’ acceptance of community DOT providers.

**Methods**

The study included 62 patients and their respective DOT providers and they were interviewed at their residences by the trained people using semi-structured interview schedules.

**Results**

Among patients, males outnumbered females by a ratio of more than ten to one, but among the DOT providers the male to female ratio was 2:1. Out of 62 patients, 50 were treated with Category I, nine with Category II and three with Category III regimens. DOT providers consisted of 37 community members and 25 family members. Regarding treatment adherence, 95% of the 62 patients had taken >75% of the drugs and 39% had taken 100% of the drugs in the regimen.

**Conclusion**

DOTS was appreciated both by the patients and their DOT providers since it is cost-effective and patient-friendly. Inadequate knowledge of DOT providers on tuberculosis and its treatment was the main cause for their poor motivation.

**Understanding stigma in the life of people living with HIV – A study from Chennai, south India**

**Background**

The nature and intensity of AIDS stigma are shaped by the social construction of the epidemic in different locales. Stigma therefore needs to be discussed in its cultural context.

**Aim**

To understand the stigma among HIV-positive individuals enrolled in controlled clinical trials on HIV/TB by the Centre.

**Methods**

The study sites were the Tuberculosis Research Centre and the STD outpatient clinic of the Government General Hospital, Chennai. Two hundred HIV-seropositive individuals were interviewed using a semi-structured interview schedule. Standardised

<table>
<thead>
<tr>
<th>Reasons*</th>
<th>Total (n=186)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Symptoms not severe</td>
<td>94</td>
</tr>
<tr>
<td>Pressure of work</td>
<td>47</td>
</tr>
<tr>
<td>Lack of money</td>
<td>85</td>
</tr>
<tr>
<td>Lack of transport</td>
<td>16</td>
</tr>
<tr>
<td>Indifference</td>
<td>48</td>
</tr>
<tr>
<td>Dependence on alcohol/drugs</td>
<td>11</td>
</tr>
<tr>
<td>Domestic preoccupation</td>
<td>19</td>
</tr>
<tr>
<td>Dissatisfaction</td>
<td>17</td>
</tr>
<tr>
<td>Others</td>
<td>37</td>
</tr>
</tbody>
</table>

* Respondents gave multiple reasons

Table 1: Reasons given by chest symptomatics for not seeking medical advice
scales were used to measure stigma and quality of life. Narrative summaries were taken down to elicit qualitative data.

Results
This study brings out the finding that the actual stigma experienced among those infected with HIV is much less (26%) as compared to the fear of being stigmatised or of perceived stigma (97%). Internalising of stigma was found to have a negative impact on the quality of life.

Conclusion
Individuals who did experience actual stigma seemed more determined to live and enjoy an above moderate quality of life. The implication of this study encourages HIV-infected individuals to rise above the stigma, avoid internalising their stigmatised feelings and work towards a better quality of life. Health providers need to address these issues in their care for HIV-infected individuals.

Status of AIDS orphans
Background
As the AIDS epidemic progresses, there is a shift from focusing on the individual to the family. Often described as a family disease, the AIDS pandemic is leaving millions orphaned in its wake. The concept of AIDS orphans has therefore gained a different meaning with reference to a child who has lost one or both parents to AIDS. In the Indian context, little is known about what happens to these children.

Aim
This study was planned to assess the status of children orphaned by the death of one or both parents to AIDS.

Methods
This was a descriptive study about the status of 140 AIDS orphans belonging to 67 families who had lost one or both parents due to AIDS. A questionnaire was used to elicit information from the surviving parent or care giver.

Results
Twenty-six percent had lost both parents to HIV/AIDS and 50% of the surviving parents were seropositive. Ten percent of the children were seropositive, while the sero status of 41% was not known. Fifty-six percent were cared for by their widowed mothers, while 21% were cared for by grandparents, usually maternal.

Conclusion
This study revealed that the main care givers of the AIDS orphans were their grandparents, mainly maternal.

Studies in Progress
Social support as perceived by patients taking treatment under RNTCP
The major public health problem of tuberculosis has a sociological dimension. Compliance to the prescribed regimen is influenced by factors like social and economic background, the patient’s acceptance of health care providers and the social support received by them. Information obtained will help us develop appropriate health education strategies to ensure timely care seeking. This study has been undertaken:

- To study the care-seeking behaviour of patients and the factors contributing to delay in diagnosis;

- To determine the financial, physical and emotional support received by them and their perceptions on DOT.

Coping strategies for tuberculosis in households of patients
Tuberculosis patients and their family members are put to a lot of inconvenience by the disease. These patients have to make a lot of adjustments and rearrangements to cope with the disease physically, emotionally, economically and socially. It is expected that the study outcomes could be used to modify motivation contents and strategies like behaviour...
therapy, psychosocial adjustment, etc, in order to ensure better compliance to treatment. A study is being undertaken to elicit the coping strategies / mechanisms which patients adopt due to tuberculosis. Patients attending the Centre and its subcentres, and those who are in their first month of treatment form the study population. It is aimed to interview 100 respondents. A semi-structured interview schedule is being used for data collection.

Social stigma and tuberculosis
Stigma is a major problem for tuberculosis patients and health care providers. Stigma may prevent persons with symptoms from coming forward for diagnosis and impair their ability to access care and comply with the prescribed treatment. The existence and the extent of stigma in relation to tuberculosis is not adequately documented. An insight into the stigma-related problems faced by patients will help health planners to develop appropriate IEC materials for the patients and the community to address this issue.

The aims of the study are:

a) To find out the perceptions of the patients and the community on tuberculosis;

b) To assess the attitude of the community towards tuberculosis patients; and

c) To document the experiences of patients with neighbours and community with reference to the stigma.

This study is being conducted in one urban (Chennai) and one rural (Kancheepuram) TU. Four hundred and fifty patients, who have been registered for treatment from January to March 2004, form the study population. The patients are being interviewed by medical social workers after two months of start of anti-tuberculosis treatment. A semi-structured questionnaire is being used to collect the necessary information.

Does alcoholism have an impact on the compliance of tuberculosis patients?
Alcoholism is one of the most common psychiatric disorders, frequently accompanied by other substance abuse disorders, anxiety and mood disorders. It often goes unrecognised in a primary health care setting. A disease like tuberculosis with a long duration of treatment requires sustained cooperation from the patients for completing their treatment. Alcoholism which impairs the mood of the individuals may have a negative impact on the patient’s compliance with tuberculosis treatment. The findings will help us identify potential non-compliant patients and develop specific motivation strategies to tackle this group.

The aims of the study are:

a) To identify tuberculosis patients with drinking problem;

b) To standardise a scale for predicting compliance to treatment among patients who report consumption of alcohol; and

c) To compare the compliance of patients who consume alcohol with those who do not.

All patients who are enrolled in the current controlled clinical trial at the Centre will be questioned regarding consumption of alcohol. Those who give a history of alcohol consumption are administered the Alcohol Use Disorders Identification Test (AUDIT), developed by the World Health Organization. An attempt is being made to rate the impact of alcoholism on patients and their families by using scores. These will be administered at the start, second month and at the end of the treatment. Their compliance for treatment will be correlated with the scores obtained.

Gender differences in perceived health-related quality of life among persons living with HIV
During the last decade there has been a lot of focus on improving the quality of life of HIV-infected individuals. Quality of life is looked upon as a product of physical, social, psychological and environmental harmony of an individual. The study aims at finding out the quality of life among men and women infected with HIV/AIDS.

This is a clinic-based study on HIV-infected individuals, allocated to the controlled clinical trials on HIV/TB conducted by the Centre.
A semi-structured interview schedule and the QOL BRIEF (Quality of life - Brief) scale is used for data collection. The interim findings of this study reveal that females score significantly higher in the psychological domain than males \((p = 0.01)\) and males score significantly higher in the sociological domain than females \((p = 0.02)\). CD4 counts did not influence the quality of life among males as it did in females in all the four domains.

A study on sexuality among HIV-positive and HIV-negative males
With the advent of HIV and the primary source of transmission being through sexual intercourse, it is important to address the issue of sexuality. A previous study done at the Centre on psychosexual issues among HIV-positive and HIV-negative individuals helped gain an insight into sexuality issues among women. It was therefore felt that it is better to address this issue among men as well in order to get a broader perspective.

The study population includes HIV-positive men attending the TRC and its subcentres at Chennai and Madurai and those who are referred by non-governmental organisations networking with the Centre. The HIV-negative individuals will be those from the STD attendees and the VCTC centres in Chennai and Madurai who have been screened for HIV and found negative. A semi-structured interview schedule and a modified sexual history questionnaire will be used for data collection. Open-ended questions and narrative summaries from the individuals would serve as qualitative data.

Adherence to antiretroviral treatment among HIV-seropositive persons on treatment for tuberculosis
The introduction of antiretroviral treatment in the control of HIV/AIDS has its challenges. The government is planning to make antiretroviral drugs available for HIV-seropositive individuals free of cost. The success of antiretroviral drugs depends on the compliance of patients to treatment. Research has revealed that adherence greater than 95% is needed to achieve virological success. It is therefore of paramount importance to find out adherence patterns among those receiving antiretroviral treatment in order to plan further strategies in the management of these individuals.

A semi-structured interview schedule is being used to obtain information from the study participants and
it is proposed to hold focus group discussions among them. The interviews will be done at different time points starting from six months. The study sites are the Tuberculosis Research Centre and STD outpatient clinics attached to the Government General Hospitals in and around Chennai.

Knowledge about sexuality among adolescents: Do parents have a role to play?
The age at which the first sexual experience takes place is relatively low in India, both in the rural and the urban areas. Among boys, the mean age is as low as 12-14 years, with friends and relatives cited as sexual partners. However, not much data is available for the girls. The influence of peers on adolescence has been well recognised. The role of parents on matters related to sexuality with their children in the teenage group (13-19 years) has not been addressed in India. A study is being carried out to determine the awareness on issues related to sex and sexuality amongst adolescents, and the role of parents in providing this information.

Knowledge, attitude and practices of persons at risk in relation to a future HIV-vaccine trial
A safe and effective HIV-vaccine offers the best hope of stopping the worldwide spread of HIV. It is very important to understand the unique barriers to vaccine readiness and the efforts that are needed to prepare the vulnerable groups. Hence, it is proposed to conduct a sociological study to find the HIV-vaccine readiness among the high-risk groups for future HIV-vaccine trials. The proposed cross-sectional study incorporates a qualitative and quantitative approach. The study population will consist of 500 persons who perceive themselves to be at risk of HIV infection due to unprotected sex and intravenous drug use.
Studies Completed

Trends in initial drug resistance over three decades in a rural community in south India

Background
The magnitude of drug resistance in tuberculosis patients and changes in it over time are useful indices for understanding the extent of resistant bacterial transmission and for monitoring the effectiveness of drug regimens in the area treatment programme. This has important implications for TB control programme.

Aim
To assess the trend of initial drug resistance over a period of three decades in a rural community in five panchayat unions in Chingleput district in south India.

Methods
A total population survey of tuberculosis in the area was undertaken in 1968-70, comprising radiographic examination of all individuals aged 10 years or more and sputum examination of those with abnormal shadows. Subsequently, the total population survey was repeated on six occasions at intervals of 2.5 years along with new entrants found at each survey, and on two more occasions (1991-92, 1994-96) in a subset of two panchayat unions. Prevalence cases and (new) incidence cases of culture-positive tuberculosis were identified in each survey, and their susceptibility to isoniazid and streptomycin was determined.

Results
Between 1968 and 1986, initial drug resistance to isoniazid increased from 12.5% to 20.7% in prevalence cases, at an average annual rate of 3.1%. For streptomycin, the increase was from 6.4% to 12.1%, at the rate of 4.9% per annum. In incidence cases, the corresponding annual rate of increase was 3.8% for isoniazid and 7.4% for streptomycin. In the subset of the population that was surveyed in 1991-92 and 1994-96, there was some evidence of a decline in the proportion of resistant cases after 1984-86 (Table 2).

Conclusion
There was a steady increase in the magnitude of initial drug resistance in the community between 1968 and 1986, which probably indicates an unsatisfactory tuberculosis programme during that period.

Association of initial tuberculin sensitivity, age and sex with the incidence of tuberculosis in south India: a 15-year follow-up

Background
A double-blind randomised controlled trial was initiated in 1968 in about 280,000 subjects in rural south India to assess the protective efficacy of the BCG vaccination. In this trial, all individuals aged ≥1 year were tested with three international units (IUs) of PPD-S and 10 units of PPD-B, derived from M. intracellulare. Case detection during the 15-year follow-up
was done using several methods (periodic population surveys, more frequent follow-up of subjects with abnormal radiograph or chest symptoms, passive surveillance through a special health centre). Radiographic and sputum coverage was consistently high. Using this exceptional data set, the prognostic importance of initial tuberculin sensitivity, age and sex have been studied, singly and together.

**Aim**
To determine the association of initial tuberculin sensitivity, age and sex with the development of tuberculosis.

**Methods**
A 15-year follow-up of 280,000 subjects in south India, where new cases of tuberculosis were detected mainly by periodic population surveys. Lifetable technique was employed to estimate tuberculosis incidence and disease risk in survivors. The independent effect of tuberculin sensitivity, sex and age at intake was determined using Cox’s proportional hazard model.

**Results**
Taking subjects with reaction size 0–7 mm to 3 IU PPD-S as reference group, the adjusted relative risk (RR) for developing culture-positive tuberculosis was 1.1, 1.9, 2.9, 3.6 and 3.3 for those with indurations of 8–11, 12–15, 16–19, 20–24 and ≥25 mm (p ≥ 0.01). Considering subjects aged 0–4 years as reference group, the adjusted RR for the other groups increased from 1.7 to 10.8 (p ≥ 0.01). Males had a substantially higher incidence (adjusted RR 3.0, p ≥ 0.001). The risk of culture-positive tuberculosis over 15 years in survivors was 3.3% (5.0% in males and 1.6% in females), and increased substantially with tuberculin sensitivity at intake. In those with >12 mm at intake, the approximate lifetime risk was 6.1% (8.6% in males and 3.1% in females) (Table 3).

**Conclusion**
Our study has shown that there was a graded relationship between the initial sensitivity (to PPD-S) and the incidence of culture-positive/smear-positive tuberculosis (r = 0.9), with incidence increasing by 9% for every mm increase in sensitivity. The rate of increase was the same for the two sexes, but decreased with age. Males had a significantly higher risk than females in every PPD-S group and the overall risk was three-fold higher.

**Mortality among a cohort of tuberculosis patients treated under RNTCP in an urban area**

**Background**
Mortality is an important epidemiological index. Reliable data on tuberculosis mortality is currently not available from India. In this study, mortality among a cohort of tuberculosis patients registered

---

**Table 2**

<table>
<thead>
<tr>
<th>Period of survey</th>
<th>Total patients</th>
<th>Percentage of patients with resistance to the following drug(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Isoniazid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Obs.</td>
</tr>
<tr>
<td>1968-70</td>
<td>689</td>
<td>12.5</td>
</tr>
<tr>
<td>1971-73</td>
<td>693</td>
<td>18.5</td>
</tr>
<tr>
<td>1973-75</td>
<td>755</td>
<td>21.1</td>
</tr>
<tr>
<td>1976-78</td>
<td>855</td>
<td>21.2</td>
</tr>
<tr>
<td>1979-81</td>
<td>790</td>
<td>19.9</td>
</tr>
<tr>
<td>1981-83</td>
<td>832</td>
<td>21.4</td>
</tr>
<tr>
<td>1984-86</td>
<td>733</td>
<td>20.7</td>
</tr>
<tr>
<td>1999-01</td>
<td>507</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>(442)</td>
<td>(9.7)</td>
</tr>
</tbody>
</table>

Obs. = Observed; std = Standardised for sex and age; N.A. = Not available. Figure in brackets is the prevalence based on the random sample survey, while the other is based on all patients tested.
in an urban area (Chennai Corporation) in the year 2000 and treated under RNTCP is reported.

### Aim

The primary objective of this study was to collect reliable mortality data from the tuberculosis patients in an urban area (Chennai). The secondary objective was to identify high-risk groups for mortality among tuberculosis patients.

### Methods

All the patients registered in the year 2000 in the Chennai Corporation from August 2002 to December 2003 were followed up for a period of 20 months from the date of start of treatment. Number of deaths occurring per 1000 persons – years of follow up is the mortality rate. This mortality rate was indirectly standardized against the mortality rate among general population to obtain the standardised mortality ratio (SMR).

### Results

Out of 3562 patients followed up, 2422 (68%) were survivors, 252 (7%) were dead and 888 (25%) had migrated. The SMR for this cohort of tuberculosis patients was 6.5 (5.7 - 7.4). SMRs for younger patients (15.5), males (7.2) Category – I (8.2), Category - II (12.0), treatment defaulters (15.6), treatment failures (19.9) and smoker-cum-alcoholics (12.0) were high and these subgroups can be regarded as high risk groups for mortality.

### Conclusion

This cohort of tuberculosis patients had 6.5 times
excess mortality than the general population. Young age, male sex, smear positivity, treatment defaulters, treatment failures and smoking-cum-alcoholism among males are all identified as high risk factors for tuberculosis mortality.

**Studies in Progress**

**Community survey of tuberculosis disease and infection**
The Centre is conducting disease surveys and tuberculin surveys to estimate the prevalence of TB infection and disease in a DOTS implemented area in Tiruvallur district of Tamil Nadu, covering a population of 5,80,000. The aim of these surveys is to study the trends over time of infection and disease and thereby to measure the epidemiological impact of implementation of DOTS strategy. The data from the baseline survey (1999-2001) showed that the prevalence of culture-positive and smear-positive tuberculosis was respectively 605 and 323/100,000 population. Annual risk of tuberculosis infection in children aged less than 10 years was 1.6% and was unaffected by sex. The disease surveys and tuberculin surveys are repeated every 2.5 years.

The first re-survey was completed in December 2003. During the year, 1,01,248 subjects were expected to be covered and we have covered 92,727 (92%) subjects for disease survey. So far, 443 cases have been diagnosed. In the second annual risk of tuberculosis infection survey, 24,928 of 26,628 children aged 0-9 years have been covered.

The second re-survey was started in January 2004. So far, of the 11,765 eligible persons, 11,113 (95%) have been covered for screening and 29 cases have been detected.
Studies Completed

Lot quality assurance sampling of sputum AFB smears stored under ambient conditions for 2 to 15 months for assessment of quality of reading in microscopy centres

Background
Direct sputum smear microscopy remains the main tool used by National Tuberculosis Control Programmes in low income high tuberculosis prevalence countries. It is imperative that the control programmes ensure the quality of the AFB sputum smear microscopy services provided by these laboratories through appropriate quality control measures. The present study documents the experiences gained using lot sampling of sputum smear for AFB to assess the quality of smear reading in a tuberculosis unit of Tiruvallur district, Tamil Nadu, during the period May 2002 to April 2003.

Aim
To assess the quality of smear reading in microscopy centres adopting lot quality assurance sampling (LQAS) of smears, stored under ambient conditions (temperature 25 – 40°C; humidity – 25 to 100%) for 2 to 15 months.

Methods
Twelve microscopy centres performing sputum AFB microscopy in a tuberculosis unit and a national reference laboratory were included in this study. In each microscopy centre, 96 slides were selected systematically among the slides prepared during a 12-month period (between May 2002 and April 2003) and checked by controllers at a national reference laboratory in July 2003. The results were matched with the results of microscopy centres. An umpire checked the discrepant smears after re-staining.

Results
Two laboratory technicians had two errors each and four had one error each; the remaining five had no errors. The controllers had seven errors.

Conclusion
LQAS of sputum smears stored under ambient conditions for 2 to 15 months was found to be suitable for assessing the quality of reading in microscopy centres.

Comparison of Ziehl-Neelsen (ZN) and auramine phenol (AP) staining methods to detect AFB in direct and deposit smears prepared from sputum samples transported in cetyl pyridinium chloride (CPC)

Background
In drug resistance surveys, sputum samples are collected in CPC-NaCl solution and transported to central laboratories for bacteriological examination. Due to unknown reasons the rate of detection of AFB in direct smears is significantly reduced using AP and ZN staining methods.
Aim
The aim of this study was to stain the direct and deposit smears.

Methods
Smears were prepared from 583 samples collected in CPC and their results were compared with culture results.

Results
Smear results were compared with the culture results and are shown in Table 4.

Conclusion
Washing the deposits of sputum samples preserved in CPC with water increased the detection rate of AFB in AP than in ZN methods.

Studies In Progress

Monitoring of the DOTS programme
Directly Observed Treatment, Short course, a global strategy for control of tuberculosis, is being implemented in India in a phased manner since 1997. Epidemiological impact of this strategy in high burden countries is not known. To understand this, the Tuberculosis Research Centre is undertaking an epidemiological impact study in five blocks of Tiruvallur district, Tamil Nadu. This is the same area where the BCG trial was done and epidemiological data on tuberculosis is available in this area prior to DOTS implementation. The Tamil Nadu government is implementing the programme and TRC is monitoring the same. The project has technical support from WHO and financial support from USAID. The project was started in May 1999. The Centre is involved in:

a) Training;
b) Monitoring of the programme;
c) Epidemiological survey of tuberculous disease and infection;
d) Bacteriological and molecular epidemiological studies;
e) Operational research.

During the period under review (April 2003 – March 2004), 883 patients have been initiated on treatment for tuberculosis in the project area covering a population of 580,000. Of these, 425 were new sputum smear positive, 228 new smear negative, 97 extra pulmonary and 133 re-treatment patients. Annualised case detection rate for new smear positive patients during the period was 86%.

Drug susceptibility profile of patients detected in DOTS demonstration and training project area, Tiruvallur
The DOTS demonstration and training centre where the epidemiological impact is being measured has 17 peripheral health institutions where tuberculosis patients are diagnosed and started on treatment. Two sputum samples were collected from these patients for culture and susceptibility tests. Among 1713 newly diagnosed patients, 89.8% had fully susceptible organisms and the prevalence of MDR-TB was 1.5%.

### Table 4

<table>
<thead>
<tr>
<th>Culture result</th>
<th>Direct ZN</th>
<th>Direct AP</th>
<th>Deposit ZN</th>
<th>Deposit AP</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pos Neg</td>
<td>Pos Neg</td>
<td>Pos Neg</td>
<td>Pos Neg</td>
<td></td>
</tr>
<tr>
<td>Pos</td>
<td>159 182</td>
<td>216 125</td>
<td>216 125</td>
<td>249 92</td>
<td>341</td>
</tr>
<tr>
<td>Neg</td>
<td>12 230</td>
<td>15 227</td>
<td>14 228</td>
<td>16 226</td>
<td>242</td>
</tr>
<tr>
<td>Total</td>
<td>171 412</td>
<td>231 352</td>
<td>230 353</td>
<td>265 318</td>
<td>583</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>47% 63%</td>
<td>63% 73%</td>
<td>63% 73%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>95% 94%</td>
<td>94% 93%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPV</td>
<td>0.92 0.93</td>
<td>0.93 0.93</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPV</td>
<td>0.56 0.64</td>
<td>0.64 0.71</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Among 459 re-treatment cases, 12.2% had MDR-TB and 61.9% had fully susceptible organisms.

In the community survey (first re-survey), 229 patients were detected and the drug susceptibility profile was MDR-TB 3% and drug susceptible TB 90%.

Involving private practitioners in the RNTCP
A feasibility study to involve private practitioners was initiated. After listing all the private laboratories in the area, 20 laboratory technicians (LTs) from the private labs were trained. All the allopathic medical practitioners in that area were given orientation in RNTCP on two occasions followed by focus group discussions to formulate the study design. A total of 48 private practitioners were enrolled. Of 681 chest symptomatics referred, 172 were diagnosed to have tuberculosis and 159 have been started on anti-tuberculous treatment under RNTCP in the Model DOTS project area.

Assessing relapse among Category I patients who have successfully completed treatment
Sputum samples from patients who have successfully completed their treatment from the project area were collected to assess relapse under the programme. Sputum specimens were collected at 12, 18 and 24 months and transported to the Centre. Specimens were examined by smear and culture. Relapse is defined as two smears positive and/or two cultures positive or one smear and one culture positive. Drug susceptibility pattern at the time of failure and relapse were also done to study the emergence of drug resistance.

Of the 534 cured patients, 503 (94%) were followed up for 18 months after completion of treatment. Of these, 62 (12%) relapsed during the 18-month period; 48 (77%) of the 62 relapses occurred during the first 6 months of follow-up. Patients who took treatment irregularly were twice more likely to have a relapse than compliant patients (20% vs 9%; adjusted odds ratio [AOR] AOR=2.5; 95% CI=1.4–4.6). Other independent predictors of relapse were initial drug resistance to isoniazid and/or rifampicin (AOR 4.8; 95% CI=2.0–11.6) and smoking (AOR=3.1; 95% CI=1.6–6.0). The relapse rate among non-smoking, treatment compliant patients with drug-sensitive organisms was 4.8%.

Risk of tuberculosis infection and disease in different economic strata
The aim of the study is to relate tuberculosis disease rates in different socio-economic groups in the community. The study is being carried out in the same population where the disease survey is undertaken to estimate the prevalence of the disease. The second re-survey (Disease Survey) was started in December 2003. All the households in a village included in the survey are being visited. The head of the family or the informant is identified and the purpose of the survey is explained to him/her and his/her cooperation is sought for the interview using the semi-structured questionnaire. Data collection was started in February 2004.

Management of MDR-TB patients from project area
Patients identified to have MDR-TB are being treated using individually tailored regimen. So far, 63 patients have been started on treatment.

Timing and its significance in the diagnosis and treatment of tuberculosis in disease endemic countries: the interplay of health seeking and health systems
A cross-sectional study was planned to quantify delay and drop out in the tuberculosis diagnostic process, to identify factors associated with delay, and to evaluate its economic and health impact in three disease endemic countries (India, Peru and Zambia). The study is being conducted in Tiruvallur district, Chennai Corporation and Kancheepuram district. A pre-coded structured questionnaire is used to collect data from tuberculosis patients and chest symptomatics by an interview schedule.

Altogether, 240 new smear positive patients, 108 smear negative, 60 extra-pulmonary tuberculosis, 20 paediatric tuberculosis patients and 1000 chest symptomatics were interviewed. To collect qualitative data, we conducted nine focus group discussions. The participants were LTs, STLS, health visitors, laboratory assistants, VHNs, treatment organizers and health inspectors.
Bacteriological and molecular epidemiological studies – Laboratory analysis of *M. tuberculosis* by RFLP

To understand the molecular epidemiology in the area and to monitor the same over time, the Centre is undertaking RFLP studies on positive cultures obtained from patients started on treatment and those isolated in the survey.

Pvu II-IS6110 and Alu I-repetitive DR RFLP analysis have been carried out in *M. tuberculosis* clinical isolates earlier. During the last year, the same isolates were subjected to an additional typing method by using a third probe, called polymorphic GC repeat sequence (PGRS). All the strains with low copies of IS 6110, were typed by this method. RFLP has been performed so far, using three different probes on a total of 1050 DNA samples of *M. tuberculosis* isolates from the project area.

Public-Private Partnership

Public-Private Partnerships in TB Control—the REACH model

The rapid expansion of the RNTCP has brought a major portion of the country under the umbrella of the DOTS programme. However, private practitioners still manage a large proportion of the unreported tuberculosis cases. To ensure complete coverage of all segments of society, it is necessary to involve the private sector also. The TRC in collaboration with ACT (Advocacy for the control of Tuberculosis), a division of REACH, a Chennai-based NGO, is testing a model for promoting public-private partnerships in TB Control. The Corporation of Chennai, large private hospitals and individual private practitioners along with REACH and TRC are the major stakeholders in this project. REACH was recently funded by the GFATM (Global Fund for AIDS, Tuberculosis and Malaria) to carry out some of the activities under this project.

The REACH model seeks to create awareness among private providers about effective TB control and induct private practitioners into the RNTCP. Further, it is identifying modalities of linking private doctors with the public health care system and determining ways to upscale such a programme.

REACH has held several workshops to sensitise private practitioners about the RNTCP, provided training and encouraged them to follow the guidelines to achieve the targets recommended by the programme. It has produced user-friendly
documents that summarise the diagnostic approaches and outline the treatment recommended by the RNTCP. A network of social workers assist private practitioners in ensuring patient compliance and maintenance of records. A major success of the project has been the identification of numerous DOT providers from the community who have volunteered their services for the programme. REACH, in collaboration with TRC, has trained 45 laboratory technicians from the private sector on all aspects of smear microscopy and enrolled private laboratories to participate in a laboratory quality control programme.

This project has shown that implementation of DOTS in the private healthcare sector is feasible. Over 100 REACH private practitioners now follow a common algorithm for diagnosis and use sputum microscopy as the primary diagnostic method. They have correctly categorized more than 1200 patients registered so far according to RNTCP guidelines. Cure, completion and default rates achieved in this project are comparable with the national programme.

The project is now exploring ways of expanding this venture, building awareness in the community by using different media and integrating the efforts of the private sector with the RNTCP.
Studies Completed

Reduced exercise capacity in non-cystic fibrosis bronchiectasis

Background
Bronchiectasis not due to cystic fibrosis (CF) is usually a consequence of severe bacterial or tuberculous infection of the lungs, which is commonly seen in children in developing countries. Though exercise testing has been used to evaluate work capacity in children with cystic fibrosis, this has not been studied for children with non-CF bronchiectasis.

Aim
To measure the cardio-respiratory response to exercise in children and adolescents with bronchiectasis, to assess the nature and severity of the functional abnormality and correlate it with resting lung function measurements.

Methods
Seventeen children (7 to 17 years of age) with clinical and radiological evidence of bronchiectasis of one or both lungs were studied at the cardio-pulmonary unit of the Centre. Pulmonary function tests, including spirometry and lung volume measurements, were performed. Incremental exercise stress test was done on a treadmill, and ventilatory and cardiac parameters were monitored. Control values were taken from a previous study.

Results
Children with bronchiectasis had lower forced vital capacity (FVC) (1.1 ± 0.4 L versus 1.5 ± 0.4 L, p=0.003) and FEV1 (0.95 ± 0.2 L versus 1.4 ± 0.3 L, p<0.002) compared to age- and sex-matched healthy controls. The patient group had significantly higher residual lung volumes (0.7 ± 0.3 L versus 0.4 ± 0.1 L, p<0.02). At maximal exercise, they had lower aerobic capacity (28 ± 6 ml/min/kg versus 38.5 ml/min/kg, p<0.0001) and maximal ventilation (24 ± 8 L/min versus 39 ± 10 L/min, p<0.001). At maximal exercise, while none of the controls desaturated, oxygen saturation fell below 88% in eight of 17 patients (Table 5).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (SD)</th>
<th>Range</th>
<th>Mean (SD)</th>
<th>Range</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V0₂ max (ml/kg/min)</td>
<td>28.1 (5.8)</td>
<td>17-38</td>
<td>37.8 (4.8)</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>179 (14.6)</td>
<td>50-200</td>
<td>207 (8.5)</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RR (breaths/min)</td>
<td>63 (5)</td>
<td>50-88</td>
<td>63 (4)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Vemax (L/min)</td>
<td>24 (7.8)</td>
<td>9.6-37</td>
<td>39 (9.8)</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VE/VO₂</td>
<td>42.4 (5.6)</td>
<td>28-50</td>
<td>40.1 (5.6)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>O₂ Sat(%)</td>
<td>88 (5.6)</td>
<td>77-98</td>
<td>92 (4.5)</td>
<td></td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Table 5
Cardiorespiratory parameters at maximal exercise
Conclusion
The findings of the study showed that children and adolescents with non-CF bronchiectasis have abnormal pulmonary function and reduced exercise capacity, which correlated with the severity of lung function abnormality. This is likely to interfere with their life as well as future work capacity. Efforts should be made to minimise lung damage in childhood by ensuring early diagnosis and instituting appropriate treatment of respiratory infections.

Evaluation of microplate alamar blue assay for drug susceptibility testing of *Mycobacterium avium* complex isolates

Background
*M. avium* complex (MAC), which includes *M. avium* and *M. intracellulare*, are the most frequent isolates among non-tuberculous mycobacteria found in clinical specimens. In patients with advanced stages of HIV-infection, disseminated MAC infection was shown to be the most common mycobacterial infection prior to MAC prophylaxis and the highly active antiretroviral treatment regimens. All wild-type MAC isolates are susceptible to macrolides (clarithromycin and azithromycin). Susceptibility testing is necessary for initial isolates to establish baseline minimum inhibitory concentration (MIC) values and for isolates obtained during treatment to detect emergence of resistance. The BACTEC radiometric method, the non-radiometric system mycobacterial growth indicator tube (MGIT) and other susceptibility testing methods, have not been used for routine testing, due to cost considerations. Microplate alamar blue assay (MABA), a colorimetric drug susceptibility testing method that uses a redox can be read visually without the need for expensive instruments.

Aim
This study investigated the reliability of MABA for susceptibility testing of MAC to clarithromycin by comparing the results with those obtained by the BACTEC radiometric method.

Methods
Fifty-one clinical isolates and five clarithromycin-resistant mutants of MAC were tested for their susceptibility to clarithromycin by MABA. The susceptibility results were compared with the results obtained by the BACTEC method.

Results
All clinical isolates were susceptible, while all mutants were resistant to clarithromycin by BACTEC. Eighty-six percent of the clinical isolates were susceptible by MABA, and one of the resistant mutants was misclassified as susceptible by this method. The overall agreement between MABA and BACTEC was 86% (Figure 1).

Conclusion
MABA is useful in drug susceptibility testing of MAC.

The bactericidal activities of Moxifloxacin and Gatifloxacin in various combinations with standard drugs against stationary phase *M. tuberculosis* Background
TB an important aspect in the treatment of tuberculosis is shortening the duration of the

![Figure 1](image-url)

**Clarithromycin minimum inhibitory concentrations**

Table showing the minimum inhibitory concentrations (MICs) of *Mycobacterium avium* complex isolates obtained by BACTEC and microplate alamar blue assay (MABA). All values are in µg/ml. The vertical line separates the National Committee for Clinical Laboratory Standards break-points for BACTEC method (MIC of ≥64 µg/ml). The horizontal line separates the interpretive breakpoint for MABA MICs based on breakpoint recommended for BACTEC.
Fluoroquinolones, especially newer 8-methoxy derivatives, moxifloxacin (MX) and gatifloxacin (GAT), demonstrated a promising mycobactericidal activity in vitro and in vivo, with a favorable pharmacokinetic profile and bioavailability of almost 96%.

Aim
The study has been carried out to determine the bactericidal activity of GAT alone and in combination with isoniazid (INH) and rifampicin (RMP) on both exponential and stationary phase cultures of M. tuberculosis H37Rv.

Methods
In vitro experiments have been carried with exponentially growing and stationary phase culture of M. tuberculosis and exposed at normal and acid pH, to the various drugs, alone or in combination. The bactericidal activity was measured at various time points by measuring the colony forming units (cfu) on 7H11 agar plates.

Results
On exponential phase culture:
Two concentrations of drugs (GAT1 and GAT2, MX1 and MX2, etc.) were tested. In the log phase culture, bactericidal activity was INH > GAT2, GAT1 and ofloxacin (OFX) > RMP, GAT, at both concentrations, augmented the bactericidal activity of INH. The combinations of GAT1 and GAT2 with RMP did not increase the activity of RMP alone (Figure 2a). MX2 showed higher activity than MX1 and OFX1, but similar to that of OFX2 at two days and then reduced in its activity resulting in 0 cfu on the fourth day. MX2 in combination with INH was as bactericidal as INH and RMP combination, resulting in 0 cfu in six hours. Even MX1 had greater activity with INH which resulted in 0 cfu at 12 hours. Hence, MX at both concentrations, were more bactericidal in combination with INH (Figure 2b).

On stationary phase culture:
RMP showed the highest activity, with counts decreasing throughout the experiment. During the first two days, GAT2 was the most bactericidal, followed by INH, GAT1 and OFX. However, none of these four drugs was bactericidal during the succeeding four days. This suggests that the stationary culture contained two bacterial populations: a majority containing bacilli that had relatively high metabolism with occasional multiplication, and the remainder with truly static, low metabolism bacilli.

The most bactericidal combination was INH + RMP, whose activity continued after day-2. GAT in combination with RMP, had in fact shown a slight antagonism at lower concentration and a marginal increase in activity during the first two days only, when GAT2 was added to RMP (Figure 3a).

As single drugs, MX2 showed better activity than other anti-tuberculosis drug tested, especially RMP,
The results of the 3-drug and 4-drug combinations showed similar kill as the control RMP/PZA/INH combination and by the RMP/PZA/MOXI and the RMP/PZA/GATI combinations. The 4-drug combinations of INH/RMP/PZA + either quinolone showed the most bactericidal activity (Figure 4). The three trend lines have closely similar slopes. The

which showed the potential of MX as a sterilising drug and that it was also better than other quinolones such as OFX. In combinations with RMP, MX2 showed similar sterilising activity like INH and RMP on day one and then it was slightly reduced with difference of 0.7 log on the sixth day. The addition of moxifloxacin even at lower concentration to RMP increased the activity of RMP on the sixth day (Figure 3b).

At acidic pH, there was rapid fall in counts during the first four days, followed by a slower kill later (during days 4 – 21), indicating that there might be two distinct subpopulations present in the culture as in patients.

The corresponding comparison between the control RMP/PZA/INH combination and the two combinations in which a quinolone has been added to the control combination are shown in Figure 5. The trend lines for the two combinations, with a quinolone added, have similar slopes which appear steeper than the trend for the control regimen. The use of acidic medium greatly improved the model to mimic bactericidal action of drugs during the treatment of pulmonary tuberculosis.

Conclusion
The addition of MX and GAT to the standard treatment regimen will result in appreciably reducing the duration of the treatment. In combination with other anti-tuberculosis drugs, MX has shown a greater bactericidal and sterilising activity.
Decreased bioavailability of rifampicin and other anti-tuberculosis drugs in patients with advanced HIV disease

Background
It was recently observed that HIV-infected individuals with and without tuberculosis had malabsorption of rifampicin and isoniazid based on the urinary excretion of the drugs which provided qualitative estimates of the effect of HIV-infection on the absorption of rifampicin and isoniazid. It was decided to investigate whether low urinary excretion of rifampicin and isoniazid represented poor bio-availability of the drugs, by estimating the concentrations of these drugs in blood.

Aim
To evaluate the pharmacokinetics of rifampicin and isoniazid in HIV-infected patients, with and without tuberculosis, and compare the values with pulmonary tuberculosis patients. The percentage dose of pyrazinamide and ethambutol excreted in urine over a particular time period were also determined.

Methods
The participants comprised of HIV-seronegative, pulmonary tuberculosis patients, 13 patients with advanced HIV-infection and 15 patients with HIV/TB. All the patients received rifampicin (450 mg), isoniazid (600 mg), pyrazinamide (1500 mg) and ethambutol (1200 mg) under supervision. Blood samples at different time points and urine excreted up to 8 hours were collected. The concentrations of rifampicin and isoniazid in plasma and that of pyrazinamide and pyrazinoic acid and ethambutol in urine were estimated. The isoniazid acetylator status was determined in all the study participants. Based on the plasma drug concentrations, certain pharmacokinetic variables were calculated. The percentage dose of pyrazinamide and pyrazinoic acid and ethambutol excreted in urine were also calculated.

Results
Plasma concentrations of rifampicin were significantly lower in both groups of HIV-positive patients compared to tuberculosis patients at all the time points tested (p<0.05). The pharmacokinetic parameters calculated on the basis of plasma rifampicin concentrations are given in Table 6. There was a significant decrease in mean peak concentration and exposure, accompanied by a significant increase in clearance of rifampicin in HIV and HIV/TB patients, when compared to tuberculosis patients (p<0.05). With respect to isoniazid, although the drug levels were lower in both HIV groups of patients than tuberculosis patients, at all the time points tested, the difference failed to attain statistical significance. It was further observed that the decreases were more pronounced in rapid rather than in slow acetylators of isoniazid. The excretion of pyrazinamide (and pyrazinoic acid) and ethambutol were reduced by 35% and 43%, and 48% and 19% respectively, in patients with HIV and HIV/TB when compared to tuberculosis patients. Stool examination for opportunistic enteric pathogens revealed Cryptosporidium parvum in their stool samples.

Conclusion
This study has found definitive evidence of malabsorption of anti-tuberculosis drugs, particularly rifampicin, in patients with advanced HIV-infection. The bioavailability of isoniazid is affected in rapid acetylators and absorption of pyrazinamide and ethambutol is also reduced. These findings have implications for treatment of patients with advanced HIV disease and tuberculosis. Further studies are required to assess whether increasing the dosages of anti-tuberculosis drugs can help overcome the effect...
of malabsorption in patients with advanced HIV disease.

Disposition of uric acid upon administration of ofloxacin, alone and in combination with other anti-tuberculosis drugs

Background
There are several reports that uric acid excretion is suppressed in tuberculosis patients receiving regimens containing pyrazinamide. This results in arthralgia which is probably due to hyperuricaemia caused by pyrazinoic acid, the primary metabolite of pyrazinamide. Ofloxacin is a valuable addition to the existing chemotherapy of pulmonary tuberculosis. Very little information is available on the disposition of uric acid following administration of ofloxacin together with other anti-tuberculosis drugs.

Aim
To study the clinical implications of uric acid disposition when ofloxacin was administered alone, and in combination with other anti-tuberculosis drugs.

Methods
Twelve male healthy volunteers were investigated on four different occasions with rifampicin, isoniazid, pyrazinamide and ofloxacin alone or in combination. The dosages of the drugs administered were approximately 10 mg/kg body weight for rifampicin, 35 mg/kg for pyrazinamide and 15 mg/kg body weight for ofloxacin and isoniazid. A partially balanced incomplete block design was adopted and the subjects were randomly allocated to each group. Uric acid concentration in urine samples excreted over 0-8 hours, was determined after coding the samples.

Results
Urinary excretion of uric acid when ofloxacin was given alone and in combination was estimated in the samples collected over the periods 0-8 hours. The mean urinary excretion of uric acid following administration of ofloxacin, rifampicin, isoniazid, pyrazinamide alone and in combinations have been presented in Figure 6.

There was a significant decrease in the group receiving pyrazinamide when compared to other groups. Though there was a decrease in uric acid excretion in the group receiving ofloxacin, it was not statistically

---

**Pharmacokinetics of rifampicin**

<table>
<thead>
<tr>
<th>Groups</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; µg/ml</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; hours</th>
<th>AUC&lt;sub&gt;0-∞&lt;/sub&gt; µg/ml.hrs</th>
<th>AUC&lt;sub&gt;0-t&lt;/sub&gt; µg/ml.hrs</th>
<th>CI ml/min.</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary TB</td>
<td>7.2</td>
<td>2.4</td>
<td>33</td>
<td>44.6</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>(5.5-8.9)</td>
<td>(1.7-3.1)</td>
<td>(26.7-39.4)</td>
<td>(36.3-52.9)</td>
<td>(11.8-18.2)</td>
<td>(2.1-3.9)</td>
</tr>
<tr>
<td>n=13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>3.4*</td>
<td>3.9</td>
<td>14.1*</td>
<td>21.2*</td>
<td>35.8*</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>(2.5-4.3)</td>
<td>(2.7-5.0)</td>
<td>(9.9-18.2)</td>
<td>(14.8-27.6)</td>
<td>(27.3-44.2)</td>
<td>(2.6-3.7)</td>
</tr>
<tr>
<td>n=13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV/TB</td>
<td>3.4*</td>
<td>3.6</td>
<td>16.5*</td>
<td>28.2*</td>
<td>37.3*</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>(2.7-4.2)</td>
<td>(2.7-4.5)</td>
<td>(12.4-20.6)</td>
<td>(18.1-38.4)</td>
<td>(21.4-53.2)</td>
<td>(1.7-3.5)</td>
</tr>
<tr>
<td>n=15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* denotes p<0.05; (vs pulmonary TB)
C<sub>max</sub>- peak concentration; T<sub>max</sub>- Time to attain C<sub>max</sub>; AUC-Area under the plasma vs time concentration curve; CI - Clearance; t<sub>1/2</sub> - Elimination half-life

---

The vertical bars denote standard deviation.
(∗Significant at p<0.05 when compared to ofloxacin alone)
O-ofloxacin; R-rifampicin; H-isoniazid; Z-pyrazinamide
significant. Rifampicin and isoniazid seemed to increase uric acid excretion.

**Conclusion**
Urinary excretion of uric acid was significantly decreased following administration of pyrazinamide, thereby leading to arthralgia. The incidence of arthralgia was mainly due to pyrazinamide and not due to either ofloxacin or other drugs in the treatment of pulmonary tuberculosis.

**Estimation of vitamin A levels among tuberculosis patients**

**Background**
Malnutrition is frequently observed in patients with pulmonary tuberculosis, as indicated by reductions in visceral proteins, anthropometric indices and micronutrient status. Several studies have demonstrated decreased vitamin A in blood during tuberculosis infection. Vitamin A supplementation results in a modulation of immune response in patients with tuberculosis and can reduce morbidity and mortality by enhancing immunity. Serum levels of vitamin A among patients with pulmonary tuberculosis have not been documented from south India. Before considering vitamin A supplementation during treatment for tuberculosis patients, there is a need to establish vitamin A levels in tuberculosis patients.

**Aim**
To assess the serum vitamin A levels among pulmonary tuberculosis patients before and after anti-tuberculosis treatment and compare the levels of the patients with subjects from similar nutritional status (healthy household contacts) and good nutritional status (healthy ‘normals’).

**Methods**
Serum vitamin A levels were estimated by HPLC in 47 smear positive, newly diagnosed pulmonary tuberculosis patients, 46 adult healthy household contacts and 30 healthy ‘normals’. Post-treatment estimations were performed in 37 patients who took treatment regularly and were cured of the disease.

**Results**
The mean serum vitamin A level in patients at start of treatment was 21.2 µg/dl, which was significantly lower than those obtained in household contacts (42.2µg/dl) and healthy ‘normals’ (48.1µg/dl). The mean vitamin A level in patients at end of anti-tuberculosis treatment increased to 38.9 µg/dl without vitamin A supplementation (Table 7). The difference between vitamin A concentration in patients at the end of treatment and that from the household contacts and healthy ‘normals’ was not statistically significant.

**Conclusion**
Low serum vitamin A levels among tuberculosis patients compared to their nutritionally matched household contacts and healthy ‘normals’ was observed. There was a significant increase in vitamin A levels at end of treatment even without vitamin A supplementation.

**Studies in Progress**

Alternative sputum processing method using chitin – sulphuric acid for improved diagnosis of tuberculosis by luciferase reporter phage assay

Luciferase reporter phage (LRP) assay has the advantages of rapidity and specificity, due to the use

<table>
<thead>
<tr>
<th>Patients</th>
<th>Vitamin A level (µg/dl)</th>
<th>Pretreatment</th>
<th>Posttreatment</th>
<th>Household contacts</th>
<th>Healthy ‘normals’</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=47</td>
<td>n=37</td>
<td>n=46</td>
<td>n=30</td>
<td></td>
</tr>
<tr>
<td>&lt;30</td>
<td>38</td>
<td>12</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;30</td>
<td>9</td>
<td>25</td>
<td>35</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>41</td>
<td>41</td>
<td>49.5</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Inter quartile range</td>
<td>37.5 - 45.0</td>
<td>37.0 - 44.5</td>
<td>45.0 - 53.6</td>
<td>51 - 64</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>27.0 - 59.5</td>
<td>30 - 65</td>
<td>36.5 - 63.5</td>
<td>42 - 80</td>
<td></td>
</tr>
</tbody>
</table>
of mycobacteriophage constructs with firefly luciferase (FfluX) gene. As a drug sensitivity assay, it has been found to have high specificity and it showed excellent agreement compared to the conventional assay. However, in order to produce a detectable reading using a luminometer, the sample should have at least $10^4$ organisms/ml. The presence of inhibitors and contaminating organisms in the sputum hampers the detection of tubercle bacilli by the phage. Processing of sputum specimens with 4% sodium hydroxide, a strong alkali, also can influence the adsorption and expression of phage. Hence, in order to use LRP as a diagnostic test, it is mandatory to improve the sensitivity of the assay by using a mild biofriendly agent for sputum processing. Accordingly, an alternative sputum processing method using Chitin-sulphuric acid is being investigated.

Temperate luciferase reporter phage construct phAETRC11 infecting \textit{M. tuberculosis} for diagnosis

The luciferase reporter phage (LRP) phAETRC11 constructed from the lysogenic phage che12 was tested for light production expressed in relative light units (RLU) with a clinical isolate of \textit{M. tuberculosis} and this was compared with the lytic phage construct phAE129.

The lytic phage construct, phAE129 (D29 based) emitted the maximum light output at 8-10 hours, which decreased drastically to reach the base level in 72 hours, as expected with a lytic phage LRP construct.

On the other hand, the lysogenic phage construct, phAETRC 11(Che12 based) showed one log increase in RLU every day and this steadily increased even after seven days of infection, as expected in a lysogenic phage LRP construct.

The sustained production of RLU with phAETRC11 promises to increase the sensitivity of LRP assay by increasing the integration time of the light output. Experiments are in progress to test sputum samples obtained from pulmonary tuberculosis patients with this phage construct.
Speciation of non-tuberculous mycobacteria by HPLC

After preliminary classification of mycobacteria based on a few phenotypic method of identification, detailed speciation of non-tuberculous mycobacteria (NTM) is being carried out by HPLC method. During the period of March 2003 to April 2004, a total of 301 isolates were identified. These strains were obtained from TRC study cases. Besides, NTM grown from specimens received from chronically ill referred cases from many parts of India were also included in this study.

Standardisation of antiretroviral drugs in blood by HPLC

Pharmacokinetic studies of antiretroviral drugs will form an important area of future research activities of the Centre. Such studies will be conducted to (i) assess drug interactions between antiretroviral and anti-tuberculosis drugs (ii) study absorption of antiretroviral drugs and (iii) establish pharmacokinetic profile of antiretroviral drugs in HIV-infected individuals in India. The above studies require simple and accurate methods to estimate antiretroviral drugs in blood. Experiments are in progress to standardise estimation of certain antiretroviral drugs such as nevirapine, efavirenz, zidovudine, lamivudine, stavudine and didanosine in blood by HPLC. These methods will be applied for future pharmacokinetic studies.

Predominance of exogenous re-infection in HIV/TB

Tuberculosis is the most common opportunistic infection in HIV-positive persons worldwide, as well as in India. HIV-infection is the biggest known risk factor for re-activation of tuberculosis. HIV-infected individuals are also at high risk of rapid progression of the disease after infection with *M. tuberculosis*, and may also be at higher risk of developing infection with tubercle bacilli on exposure. Recurrent tuberculosis might be due either to exogenous re-infection or endogenous re-activation. Our previous studies have shown that among non-HIV/TB patients the relapse was due to endogenous re-activation in about 69% of the cases and only 41% was due to exogenous re-infection.

Recently, among the HIV/TB patients who have had a recurrence, it was seen that exogenous infection was more predominant than endogenous re-activation. RFLP was done with IS6110, DR or spoligo and MIRU/VNTR typing on the pretreatment tuberculosis isolates, as well as the isolates which recurred after treatment.

It was seen that the recurred isolates did not match the pretreatment isolates, indicating the predominance of exogenous re-infection.
Studies Completed

Characterisation and purification of antigenic components of *M. tuberculosis* - Cellular immune response to recombinant 27kDa (mpt51, Rv3803c) in human tuberculosis

**Background**
The 27kDa (mpt 51) antigen was observed in our laboratory to be specifically recognised by tuberculosis (TB) sera, in immunoblot. Purification of the native antigen, over expression in *E. coli* and characterisation of 27kDa (mpt 51) have been described in detail in previous years' Annual Reports. Antibody response to recombinant 27kDa (r27kDa) and its diagnostic value has also been shown in previous years' Annual Reports.

**Aim**
To study the cellular immune response to r27kDa antigen in the human host, namely, TB patients and normal healthy subjects (NHS).

**Methods**
Lymphocyte proliferation test was carried out using peripheral blood mononuclear cells (PBMC) of both the TB and NHS. The PBMC of the TB (N=15) and NHS (N=10) were stimulated with polyclonal mitogen (PHA), crude mycobacterial antigen (PPD) and r27kDa. Proliferation of PBMC was measured by ³H thymidine incorporation method. Two types of cytokines were studied, which included IFN-γ, a pro-inflammatory cytokine (Th1) and IL-4, which is an anti-inflammatory cytokine (Th2). Cytokine levels were measured by ELISA.

**Results**
**Lymphocyte proliferative response**
Among the tuberculosis patients, 19 of 25 patients (76%) were responders for PPD and 5 of 25 (20%) were responders for r27kDa antigen. Among the normal healthy subjects, 23 of 25 (92%) were found to be responders for PPD and 3 of 25 patients (12%) were found to be responders for 27C and 27N antigens. The r27kDa antigen could induce lymphocyte proliferation only minimally, and in a smaller number of TB patients and NHS as compared to PPD.

**Cytokine response**
Significant increase in level of IFN-γ could be seen in PHA and PPD stimulated PBMC of TB and NHS, when compared to the control unstimulated cultures (p<0.05). However, such a significant difference was not seen in r27kDa stimulated PBMC. Also, there was no difference in IFN-γ levels between the TB and NHS (Figure 7). Similar results were obtained for IL-4 also.
Early activation marker (CD69)
CD69 expression is used as an indication that T cells have encountered antigen-presenting cells. Hence, by flow cytometric studies, stimulated PBMC were studied for the expression of CD69, in order to understand the activation potential of the stimulants.

When the PBMC activated with r27kDa antigen were studied, eight TB patients, out of 11 showed increased expression of CD69 marker in both CD4+ and CD8+ cells, when compared to the control unstimulated cells. Among them, four patients showed increased CD4+/CD69 and four had increased CD8+/CD69 expression (Figures 8a & 8b).

However, when the level of activation was studied, levels of CD4+/CD69+ and CD8+/CD69+ cells were less in TB patients than in normal healthy subjects.

Intermediate activation marker (CD25)
CD25 is an IL-2α-chain (low-affinity IL-2) receptor, induced by antigenic stimulation and necessary for the IL-2 secretion that, in turn, regulates the proliferation of the T cells. Expression of CD25 marker on PBMC was studied as an intermediate activation marker.

When r27kDa antigen stimulated NHS were analysed, there was no significant difference in cell numbers (both CD4+ and CD8+) expressing CD25 as compared to un-stimulated controls. Similar findings were seen in TB also.

Conclusion
The absence of any in vitro stimulating activity for r27kDa raises a doubt as to whether it is not equivalent to native 27kDa. Native 27kDa has been purified and it is being tested for its stimulatory activity.

Role of HLA-DR2, mannose-binding lectin (MBL), vitamin D receptor gene variants on immune functions in pulmonary tuberculosis
Regulatory role of VDR gene
Background
1, 25 di-hydroxy vitamin D₃, the active form of vitamin D₃, has been shown to modulate the immune functions. It acts through vitamin D receptor, a nuclear hormone receptor. Our earlier studies revealed the differential association of variant vitamin D receptor (VDR) genotypes of BsmI, ApaI, TaqI and FokI polymorphisms of VDR gene with the susceptibility to pulmonary TB.

Aim
In the present study, the regulatory role of variant VDR genotypes of BsmI, ApaI, TaqI and FokI
polymorphisms on the vitamin D\textsubscript{3} modulated immune functions was assessed.

**Methods**
The study was carried out in 60 normal subjects and 50 pulmonary TB patients. Macrophage phagocytosis with live \textit{M. tuberculosis}, spontaneous lymphoproliferative response, and \textit{M. tuberculosis} culture filtrate antigen (CFA) induced lymphocyte response, were studied in pulmonary TB.

**Results**
This study revealed that vitamin D\textsubscript{3} at a concentration of 1x10\textsuperscript{-7}M enhanced the macrophage phagocytosis of live \textit{M. tuberculosis} in normal subjects, who had a phagocytic index of less than 20%. This modulatory effect was not seen in pulmonary TB. Enhanced phagocytosis was observed in normal subjects with the genotypes BB (homozygotes of frequent allele ‘B’ of \textit{Bsm}I polymorphism), TT (homozygotes of frequent allele ‘T’ of \textit{Taq}I polymorphism) and tt (homozygotes of infrequent allele ‘t’ of \textit{Taq}I polymorphism).

Vitamin D\textsubscript{3} at various concentrations (1x10\textsuperscript{-9}M, 1x10\textsuperscript{-8}M, 1x10\textsuperscript{-7}M) suppressed the CFA induced lymphocyte response in normal subjects, but no suppression was observed in pulmonary TB patients. This suppression was observed in normal subjects with BB and tt genotypes (Figures 9 & 10).

Normal individuals with BBAAtt showed a significant increase in phagocytic efficiency upon vitamin D\textsubscript{3}.

---

**Figure 9**
Influence of variant genotypes of \textit{Bsm}I polymorphism of VDR gene on Vitamin D\textsubscript{3} modulated lymphocyte response

---

**Figure 10**
Influence of variant genotypes of \textit{Taq}I polymorphism of VDR gene on vitamin D\textsubscript{3} modulated lymphocyte response

---

Numbers in parentheses represent number of individuals positive for the genotypes. *NHS BB CFA vs CFA + vit D\textsubscript{3} (10\textsuperscript{-9}M): p=0.031, #NHS BB CFA vs CFA + vit D\textsubscript{3} (10\textsuperscript{-8}M): p=0.029, $ NHS BB CFA vs CFA + vit D\textsubscript{3} (10\textsuperscript{-7}M): p=0.003, + + NHS Bb CFA vs CFA + vit D\textsubscript{3} (10\textsuperscript{-7}M): p=0.007

Numbers in parentheses represent number of individuals positive for the genotypes. *NHS Tt CFA vs CFA + vit D\textsubscript{3} (10\textsuperscript{-8}M): p=0.004, $ NHS tt CFA vs CFA + vit D\textsubscript{3} (10\textsuperscript{-9}M): p=0.015, # NHS tt CFA vs CFA + vit D\textsubscript{3} (10\textsuperscript{-7}M): p=0.052, @ tt NHS CFA vs CFA + vit D\textsubscript{3} (10\textsuperscript{-7}M): p=0.015
treatment compared to bbaaTT genotype. Moreover, increased suppression of antigen induced lymphoproliferation was observed in individuals with extended genotype BBAAtt.

Conclusion
Present study suggests that the genotypes BB, tt, Ff and the extended genotype BBAAtt or the haplotype BAt may be associated with increased vitamin D receptor expression, which in turn may regulate the immunomodulatory effect of vitamin D_3_ probably through altered level of cytokines. Moreover, normal subjects positive for BB, tt and FF genotypes with higher endogenous production of vitamin D_3_ may be associated with down regulated immune functions and such individuals may be susceptible to tuberculosis.

Immune response in tuberculous pleuritis
Differential cytokine response in tuberculous pleuritis
Background
In _M. tuberculosis_ infection, a Th1 response has been postulated to be protective. There are reports based on _in vivo_ and _in vitro_ studies that yield different results on the pattern of T cell cytokines, and T helper cell response, in tuberculosis. TB pleuritis is the best model to understand the host immune response at the site of infection. Moreover, pleural fluid cells that closely encounter tubercle bacilli and generate appropriate immune response against tuberculosis, are a better source to study immunity at the site of the disease.

In our previous annual report, the role of tumor necrosis factor – alpha (TNF-α) in TB pleuritis was shown.

Aim
To understand the systemic and localised _in vivo_ immune response in TB pleuritis, the levels of cytokines involved in Th1 and Th2 type of immune response was studied.

Methods
The _in vivo_ and _in vitro_ immune response in tuberculous (TB) and non-tuberculous pleuritis patients (NTB) was evaluated. The levels of IFN-γ, TNF–α, IL-12p40 and IL-4 cytokines were measured in plasma and pleural fluid of 78 patients (47 TB and 31 NTB patients) by ELISA. In 19 TB patients, _in vitro_ cytokine levels were also measured in the supernatants of peripheral blood mononuclear cells (PBMC) and pleural fluid mononuclear cells (PFMC), stimulated with PPD, culture filtrate (CF) and heat killed _M. tuberculosis_ (MTB).

Results
The mean cytokine levels in plasma (BL) and pleural fluid (PF) of tuberculous pleuritis patients and non-TB patients are shown in Figures 11a & 11b. There was a significant increase in Th1 type of cytokines (IFN-γ, TNF-α and IL-12) in the pleural fluid of tuberculous, but not in non-tuberculous pleuritis patients. This implies that the cytokine response is of Th1 type at the site of infection in tuberculous pleuritis.
To find whether the *in vivo* pattern of cytokine response is reflected under *in vitro* conditions, IFN-γ, IL-12 and IL-4 levels in the supernatants of PBMC and PFMC stimulated with various mycobacterial antigens were measured, the results of which are depicted in Figures 12a & 12b. The supernatants of PFMC stimulated with PPD and MTB showed a significant increase in IFN-γ and TNF-α levels compared with the control (p<0.05). In contrast, IL-12 levels were high in PBMC supernatants and almost undetectable in PFMC supernatants. Interestingly, the IL-4 levels in all the stimulated conditions of PFMC were significantly higher than the control levels. This shows that *in vitro* cytokine response is of Th 0 type.

**Conclusion**

There is a differential T helper cytokine response in tuberculous pleuritis suggestive of Th1 *in vivo* and Th0 *in vitro*.

---

**Molecular and immunological characterisation of *M. tuberculosis* strains with single copy of IS6110 – Humoral immune response to sonicate antigens**

**Background**

Our Restriction Fragment Length Polymorphism (RFLP) studies have shown that the most prevalent (40%) strains of *M. tuberculosis* from south India contain a single copy of IS6110 insertion sequence. These widely spread strains of *M. tuberculosis* are of importance because of their virulence and their role in immunity. Earlier, cell mediated immune response to the sonicate antigens prepared from these prevalent strains of *M. tuberculosis* harbouring immune response to sonicate antigens was shown by us.

**Aim**

The aim of this study was to characterise the humoral immune response induced by the single copy strains.

**Methods**

The humoral immune response to these antigens was studied in normal, healthy, PPD positive subjects (n=30) and pulmonary tuberculosis patients (n=30) by ELISA and Western blot.

**Results**

In the SDS-PAGE protein profiles of 13 sonicate antigens (S1-S13), S10 and S12 showed the maximum differential protein bands in low molecular mass region of 10-30kDa. Our ELISA results showed significant increase in *M. tuberculosis* specific IgG antibodies in TB plasma for H37Rv, followed by PPD, S1 and S10 antigens. Immunoblot analysis of S10 and S12 sonicate antigens (Figure 13) showed very specific recognition pattern at low molecular mass region by TB plasma alone. The antigens S1, S2 and H37Rv showed either cross reactive or minimal recognition patterns. The percentage positivity of protein bands in TB plasma for antigen S10 ranged from 12-77% (Figures 14a & 14b). The maximum positivity was observed for 16kDa and 45kDa (77%) followed by 38kDa (66%) protein bands.

**Conclusion**

From our results, the sonicate antigen S10 was found to be discriminatory by ELISA and Western blot and is thus a good candidate for further purification of its individual proteins to be evaluated for diagnosis.
Cloning, over-expression, and characterisation of a serine/threonine protein kinase *pknI* from *Mycobacterium tuberculosis* H37Rv

**Background**

Protein phosphorylation-dephosphorylation is the principal mechanism for translation of external signals into cellular responses. Eukaryotic-like serine/threonine kinases have been reported to play important roles in bacterial development and/or virulence. The PknI protein is one of the 11 eukaryotic-like serine/threonine kinases in *M. tuberculosis* H37Rv. From the bioinformatics ...
studies, PknI protein has been shown to have an N-terminal cytoplasmic domain followed by a transmembrane region and an extracellular C-terminus suggestive of a sensor molecule.

Aim
To clone, over-express, and characterise the entire coding region and the cytoplasmic domain of PknI.

Methods
PknI was expressed as a fusion protein with an N-terminal histidine tag, and immobilised metal affinity chromatography was used for purification of recombinant proteins.

Results
The purified recombinant proteins were found to be functionally active through in vitro phosphorylation assay and phosphaamino acid analysis. In vitro kinase assay of both proteins revealed that PknI is capable of autophosphorylation and showed manganese-dependent activity (Figures 15a & 15b). Phosphoamino acid analysis indicated phosphorylation at serine and threonine residues (Figures 16a & 16b). Southern blot analysis with genomic DNA highlighted the conserved nature of pknI among the various mycobacterial species. In silico analysis revealed a close homology of PknI to StkI from Streptococcus agalactiae, shown to have a role in virulence and cell segregation of the organism.

Conclusion
PknI gene from M. tuberculosis H37Rv has been cloned and the protein has been characterised.

Disruption of response regulator gene, devR, leads to attenuation in virulence of M. tuberculosis

Background
The M. tuberculosis genome encodes 11 complete two-component systems and seven orphan signal transduction proteins. While four of them – MtrA, SenX3-RegX3, TrcS-TrcR and DevR-DevS – have been established as authentic two-component systems based on their biochemical properties, their role in regulating various cellular functions is just beginning to be elucidated. The DevR-DevS (Rv3133c-Rv3132c) system was first identified in our laboratory by a subtractive hybridisation strategy employed with the specific intention of identifying virulence genes of M. tuberculosis. The devR-devS genes encode a response regulator, DevR, and a histidine sensor kinase, DevS, respectively, that display
In vitro kinase assay and effect of divalent cations

(a) Full-length PknI
(b) Cytoplasmic domain of PknI

Phosphoamino acid analysis of PknI

(a) Full-length PknI protein (indicated by arrow head) probed with
Lane 1: Anti-phospho serine antibody;
Lane 2: Anti-phospho threonine antibody
(b) Cytoplasmic domain of PknI (indicated by arrow head) probed with
Lane 1: Anti-phospho serine antibody;
Lane 2: Anti-phospho threonine antibody
phosphorylation properties typical of two-component system proteins.

**Aim**
To demonstrate the participation of DevR in the virulence of *M. tuberculosis*.

**Methods**
A *devR:*kan mutant strain of *M. tuberculosis* was constructed by allelic exchange and characterised with respect to its morphology in laboratory media and within human monocytes, and its contribution to the pathogenesis of *M. tuberculosis* infection, was studied using guinea pigs. The mutant was complemented with a wild-type copy of the *devR* gene resulting in the reversion of dispersed culture phenotype in laboratory culture media.

**Results**
The *devR* mutant strain showed reduced cell-to-cell adherence in comparison to the parental strain in laboratory culture media. This phenotype was reversed on complementation with a wild-type copy of *devR*. The *devR* mutant and parental strains grew at equivalent rates within human monocytes both in the absence and the presence of lymphocytic cells. The expression of DevR was not modulated upon entry of *M. tuberculosis* into human monocytes. However, guinea pigs infected with the mutant strain showed a significant decrease in gross lesions in lung, liver and spleen; only mild pathological changes in liver and lung; and a nearly 3 log lower bacterial burden in spleen, as compared to guinea pigs infected with the parental strain (Table 8).

**Conclusion**
The results of this study suggested that DevR is required for virulence in guinea pigs but is not essential for entry, survival and multiplication of *M. tuberculosis* within human monocytes *in vitro*. The attenuation in virulence of the *devR* mutant in guinea pigs together with DevR-DevS being a bona fide signal transduction system indicated that DevR plays a critical and regulatory role in the adaptation and survival of *M. tuberculosis* within tissues.

HIV alters plasma and *M. tuberculosis*-induced cytokine production in patients with tuberculosis

**Background**
The pattern of cytokines produced by T lymphocytes plays a central role in the susceptibility to tuberculosis. HIV infection is associated with a profound deregulation of the immune system and alterations in the cytokine profile.

**Aim**
To test the hypothesis that HIV infection brings about an alteration in the host immune response to tuberculosis.

**Methods**
The study population comprised four groups of individuals:

(a) 29 HIV-seropositive patients with active TB;
(b) 21 HIV-seronegative patients with tuberculosis;

<table>
<thead>
<tr>
<th>Strain</th>
<th>Lung (20)</th>
<th>Liver (30)</th>
<th>Spleen (40)</th>
<th>Lymph node (10)</th>
<th>Total (100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H37Rv</td>
<td>13.75±2.5</td>
<td>26.25±7.5</td>
<td>30±14.4</td>
<td>6.5±14.4</td>
<td>77±23.2</td>
</tr>
<tr>
<td><em>devR</em> mutant</td>
<td>6±5.4</td>
<td>1.6±3.58**</td>
<td>24±13.42</td>
<td>6.8±2.68</td>
<td>38.4±12.64*</td>
</tr>
</tbody>
</table>

*p<0.05 as derived by application of the Mann-Whitney test.

a: Visual scores represent arithmetic means±S.D. from five and four animals for *M. tuberculosis* *devR* mutant and H37Rv strains respectively. The maximum score assigned for each organ is given within parentheses with a maximum body score of 100 as described.
(c) 26 HIV-seropositive patients without active tuberculosis; and

(d) 22 healthy controls.

Mycobacterial antigen-induced production and plasma levels of the inflammatory cytokine interferon-γ (IFN-γ) and its regulatory cytokines interleukin-12 (IL-12), IL-18, and IL-10 were determined in all the study participants. Peripheral blood mononuclear cells (PBMC) were obtained by density gradient centrifugation and cytokines were measured by capture ELISA. Lymphocyte subpopulations were analysed in whole blood by dual-colour flow cytometry.

Results
PBMC of tuberculosis patients with HIV infection produced lesser amounts of IFN-γ and IL-12 compared with tuberculosis patients without HIV infection after in vitro stimulation with mycobacterial antigens. There was no difference in antigen-induced IL-18 production in tuberculosis patients with or without HIV infection (Table 9).

The in vitro cytokine pattern did not correlate with that seen in vitro. Higher levels of IFN-γ, IL-12, and IL-18 were detected in the plasma of TB patients infected with HIV compared with TB patients without HIV infection. The presence of significantly higher plasma levels of pro-inflammatory cytokines suggests a greater degree of immune activation in individuals with HIV and TB, particularly those with low CD4+ counts. In vitro IL-10 production by HIV-positive tuberculosis patients was similar to that of the HIV-negative tuberculosis group and higher than in HIV-positive individuals without TB, but the plasma levels were similar.

Conclusion
Significant immune activation as seen by high plasma IFN-γ, IL-12, and IL-18, in patients infected with both TB and HIV compared with those with only one infection has been demonstrated. The Th1 arm of the immune system appeared to be maximally stimulated, particularly in individuals with advanced HIV disease, and this was likely to be a double-edged sword. HIV infection downregulates the in vitro Th1 cytokine response to tuberculosis and simultaneously increases systemic levels of these cytokines.

Influence of active tuberculosis on chemokine and chemokine receptor expression in HIV-infected persons
Background
Chemokine receptors CCR5 and CXCR4 have been identified as major co-receptors that act in combination with CD4+ surface molecules for HIV docking and entry. Interactions between chemokine receptors and their corresponding ligands could therefore block and downregulate co-receptor expression and effectively inhibit HIV life cycle.

<p>| Table 9 IL-18 levels (Mean ± SE) in plasma and culture supernatants of in vitro stimulated PBMCs |
|-----------------------------------------------|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Subjects</th>
<th>PPD-stimulated PBMC (pg/ml)</th>
<th>Hk-M.tb-stimulated PBMC (pg/ml)</th>
<th>Plasma (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>HIV + TB + (CD4 &gt; 200)</td>
<td>138.3 ± 92.7</td>
<td>204.4 ± 124.5</td>
<td>1191.6 ± 139.1*</td>
</tr>
<tr>
<td>II</td>
<td>HIV + TB + (CD4 &lt; 200)</td>
<td>78.3 ± 55.5</td>
<td>106.4 ± 20.9</td>
<td>1167.8 ± 150.5*</td>
</tr>
<tr>
<td>III</td>
<td>TB +</td>
<td>87.5 ± 26.2</td>
<td>236.6 ± 49.3</td>
<td>767.8 ± 240.0**,** ***</td>
</tr>
<tr>
<td>IV</td>
<td>HIV + TB - (CD4 &gt; 200)</td>
<td>5.5 ± 5.1</td>
<td>5.5 ± 2.9</td>
<td>635.5 ± 79.7 <strong>,</strong> ***</td>
</tr>
<tr>
<td>V</td>
<td>HIV + TB - (CD4 &lt; 200)</td>
<td>11.2 ± 0.1</td>
<td>12.5 ± 4.7</td>
<td>790.6 ± 64.5**,** ***</td>
</tr>
<tr>
<td>VI</td>
<td>Healthy controls</td>
<td>92.2 ± 25.2</td>
<td>99.1 ± 31.3</td>
<td>333.7 ± 63.5 *,<strong>,</strong> ***</td>
</tr>
</tbody>
</table>

*p < 0.01 compared with group III  **p < 0.05 compared with group I  ***p<0.05 compared with group II
Aim
To test the influence of active tuberculosis on the expression of the chemokine receptors CCR5 and CXCR4, and circulating levels of the β-chemokines, macrophage inflammatory protein-1 alpha and beta (MIP-1α, MIP-1β).

Methods
CCR5 and CXCR4 expressing CD\textsubscript{3} and CD\textsubscript{4} T cells (by flowcytometry) and plasma levels of MIP-1α, MIP-1β and RANTES (by ELISA) were estimated in 14 HIV/TB, 14 HIV and 10 TB patients and 10 healthy individuals.

Results
The proportion of total T cells, particularly helper T cells, expressing CCR5 and CXCR4 was significantly lower in HIV-seropositive individuals, irrespective of the presence or absence of tuberculosis, as compared to healthy and tuberculosis groups (Table 10).

On the other hand, plasma levels of MIP-1α, MIP-1β and RANTES tended to be higher in HIV-infected individuals than in healthy controls (Table 11).

Conclusion
Chemokine receptors CCR5 and CXCR4 were downregulated in patients with HIV and TB compared to controls.

Apoptosis of PBMCs in patients with HIV infection and disease
Background
HIV infection leads to a progressive loss of CD\textsubscript{4} lymphocytes, which results in decreased

The HIV/TB patients showed a trend towards higher production of chemokines than HIV-infected individuals. There was no statistically significant correlation between viral load and cellular expression of chemokine receptors or plasma β chemokine levels in these individuals. These data suggest a down-regulation in the in vivo expression of CCR5 and CXCR4 on CD\textsubscript{4} cells in HIV-infected individuals, with and without TB, in India. A trend towards increased production of MIP-1α, MIP-1β and RANTES was observed in HIV-infected individuals when compared to uninfected controls; among HIV-infected, those with tuberculosis had higher plasma chemokine levels those without.
general immunity and increased susceptibility to opportunistic infections and malignancies. Though apoptosis has been implicated as one of the mechanisms involved in the massive cell depletion in HIV infection, the extent of apoptosis occurring in HIV-infected patients, particularly those with TB, is not well documented.

**Aim**
To evaluate the extent of apoptosis in the PBMCs of HIV-infected patients with and without tuberculosis and test the dual signal hypothesis of cell proliferation and apoptosis.

**Methods**
PBMCs were obtained from 20 HIV patients, 10 TB patients, 20 HIV-positive TB patients and 10 healthy controls. Genomic DNA and RNA were extracted from fresh PBMCs as well as from PBMCs stimulated *in vitro* with phytohaemaglutinin (PHA), purified HIV-1 gag core protein p24 and PPD from *M. tuberculosis*. DNA fragmentation and apoptosis were evaluated by multiple techniques, including agarose gel electrophoresis, spectrometry, flow cytometry and multiplex PCR (M-PCR).

**Results**
The results of this study showed that both HIV infection and TB induce apoptosis of PBMCs (Table 12).

**Conclusion**
Patients with both diseases have increased levels of apoptosis that explains the steady decline in the immune status. A quantitative analysis of apoptosis was done by dual staining of Annexin-V and propidium iodide. The results correlated with the HIV-1 viral load and CD$_4$ counts.

**Lympho proliferative response to HIV (p24) and *M. tuberculosis* (PPD) antigens in HIV-infected individuals with and without tuberculosis**

**Background**
In the chronic stage of HIV infection, T cell proliferative responses to HIV antigens are rare and mostly of low level. The influence of opportunistic infections such as tuberculosis on these cellular immune responses to HIV and other recall antigens is not well documented.

**Aim**
To evaluate the lympho-proliferative response to HIV antigen (p24) and *M. tuberculosis* antigen PPD in HIV-infected individuals with and without tuberculosis.

**Methods**
Proliferation assays were performed on freshly isolated PBMCs from 20 HIV-TB, 20 HIV and 10 TB patients and 10 healthy individuals using PHA, purified HIV (p24) and *M. tuberculosis* PPD antigen. The CD$_4$ counts and HIV-1 plasma viral load were correlated with the lymphocyte stimulation index.

**Results**
The mitogenic response to PHA in HIV-infected individuals was significantly impaired (HIV/TB = 7.6 ± 0.9; HIV = 8.9 ± 1.4) when compared to healthy controls (50.6 ± 4.0) and tuberculosis patients (43.0 ± 5.2) (p<0.001). The proliferative response to HIV specific p24 antigen was significantly higher in the HIV-infected individuals (SI = 4.5 ± 0.5) than the

<p>| Table 12: Apoptotic peripheral blood mononuclear cells in the study population |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>Percentage of apoptotic cells (Mean±SD)</th>
<th>Freshly isolated</th>
<th>Unstimulated</th>
<th>PHA</th>
<th>p24</th>
<th>PPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV+TB+  (n= 20)</td>
<td>5.35 ± 3.3</td>
<td>39.5 ± 15.2</td>
<td>56.6 ± 9.3 *</td>
<td>7.35 ± 2.8*</td>
<td>13.9 ± 6.6</td>
</tr>
<tr>
<td>HIV+TB- (n = 20)</td>
<td>5.2 ± 2.2</td>
<td>33.5 ± 9.5</td>
<td>46.5 ± 15.4*</td>
<td>8.7 ± 5.5*</td>
<td>11.2 ± 6.2</td>
</tr>
<tr>
<td>HIV-TB+  (n = 10)</td>
<td>2.7 ± 2.3</td>
<td>19.1 ± 8.6</td>
<td>27.4 ± 10.4</td>
<td>3.3 ± 1.8</td>
<td>20.6± 14.8</td>
</tr>
<tr>
<td>Controls (n=10)</td>
<td>2.4 ± 2.6</td>
<td>13 ± 6.1</td>
<td>20.1 ± 6.1</td>
<td>2.2 ± 1.5</td>
<td>10.8 ± 5.9</td>
</tr>
</tbody>
</table>

P < 0.001 against controls, P < 0.05 against HIV+TB-
PHA - phytohaemaglutinin
HIV + TB+ HIV and tuberculosis positive
HIV + TB- HIV positive tuberculosis negative
HIV - TB+ HIV negative tuberculosis positive
HIV-negative individuals (1.4 ± 0.2) (p<0.01). *In vitro* response to PPD was significantly elevated in the HIV/TB (16.0 ± 2.2) when compared to healthy controls (8.0 ± 1.4) (p<0.01) and was maintained even in the group with low CD4 counts. The decline in CD4 counts did not significantly affect PPD response. The proliferative response to p24 showed a direct correlation with the CD4 counts (Pearson correlation r = 0.43, p < 0.01) and an inverse correlation with viral load (Pearson correlation r = -0.33, p < 0.05).

**Conclusion**

The presence of active tuberculosis did not significantly alter the proliferative response to p24 antigen in HIV-infected individuals. Lymphocyte proliferation to the HIV antigen p24 was not affected by the presence of active TB but was impaired in patients with CD4 counts < 100 per cu. mm. The response to PPD was preserved even in patients with advanced disease who have impaired responses to PHA and HIV antigens, indicating the presence of tuberculosis-specific T cells in patients in endemic area. Lymphocyte proliferation to PHA and HIV antigens was impaired in patients with HIV disease and tuberculosis but response to PPD is preserved.

**Age-related changes in blood lymphocyte subsets of south Indian children**

**Background**

Enumeration of lymphocyte subsets has been widely used for the diagnosis and monitoring of several haematological and immunological disorders. Various studies have demonstrated age, sex and racial differences in lymphocyte subset expression. Reference values are not available for Indian children and there is a need for this information to replace commonly used, but inappropriate, adult lymphocyte subset ranges.

**Aim**

To generate data on the levels of all relevant lymphocyte subsets in healthy Indian children.

**Methods**

One hundred and thirty-eight healthy children between 3 and 15 years of age, attending a local government school in Chennai, were included in the study. The subjects were categorised into six groups based on age group. Haemoglobin levels, and total and differential cell counts were determined using an automated counter, and lymphocyte subsets were analysed by flow cytometry.

**Results**

The mean percentage and absolute count of T lymphocyte subsets in different age groups of children are given in Table 13. The mean (SD) absolute lymphocyte count declined with age from 4338 (1031) at three years to reach a plateau of 3096 (914) at 11 to 13 years (p < 0.05). A significant decline was also observed in the absolute numbers of CD3, CD4,
CD\textsubscript{8} and CD\textsubscript{19} cells. However, the percentage values of CD\textsubscript{3}, CD\textsubscript{4}, CD\textsubscript{8}, CD\textsubscript{16/56} cells and the CD\textsubscript{4}/CD\textsubscript{8} ratio remained fairly stable across the age range.

**Conclusion**
This study has confirmed earlier reports on age-related changes in human blood lymphocyte sub-populations and provided some baseline data for use in India. This study data would prove useful in interpreting disease-related changes in lymphocyte subsets in Indian children of different age groups. Age-related decrease in the absolute lymphocyte count as well as numbers of CD\textsubscript{4} and CD\textsubscript{8} cells was found to occur between the ages of three and eleven years. A nomogram relating age to CD\textsubscript{4} count has been developed. These findings, of a high correlation between absolute lymphocyte count and CD\textsubscript{4} count, suggest that the former could be used as a surrogate marker where facilities for CD\textsubscript{4} testing are not available.

**Differentiation of highly prevalent IS6110 single copy strains of M. tuberculosis by restriction fragment length polymorphism**

**Background**
The TRC has reported a high percentage of single copy strains and no copy strains from an endemic urban area of Chennai, south India. When a high percentage of low copy strains are prevalent in the area, it becomes mandatory to use secondary typing methods to differentiate the low copy strains, especially the single copy strains. The most popular secondary typing methods are PGRS fingerprinting using recombinant plasmid PTBN12 and DR spoligotyping.

**Aim**
The broader objectives were to study the polymorphism of M. tuberculosis strains from the rural set up, the frequency of low copy strains in this community and to establish tuberculosis transmission by conventional epidemiology.

**Methods**
The DNA fingerprintings of M. tuberculosis strains has been prospectively analysed in a rural community from a high prevalence area in south India, with an ongoing DOTS programme. Strains from 451 culture positive cases, diagnosed during July 1999 – Dec 2000, were fingerprinted initially by both IS6110 and DR probes followed by PGRS typing only on low copy strains.

**Results**
The strains were grouped into four categories – single copy, low copy (2-5 bands), high copy (6-17 bands) and no copy based on IS6110 typing. Totally, 106 low copy strains (23%) showed 64 unique patterns and 60% of strains got differentiated in this group. In the high copy group, 151 strains showed 136 unique patterns giving high degree of polymorphism and thereby differentiating nearly 90% of the strains. Eight strains were also observed (2%) that did not show IS6110 copy (no copy group). Thus, IS6110 based RFLP showed high degree of discriminatory power among high copy number strains.

Additional DR and PGRS probes were used to differentiate the high percentage (41%) of isolates with single copy of IS6110 (Table 14).

<table>
<thead>
<tr>
<th>IS6110 single copy clusters (size of the band)</th>
<th>Total No. of strains</th>
<th>No. of DR patterns</th>
<th>No. of PGRS patterns</th>
<th>% of strains differentiated</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS6110 single copy clusters (size of the band)</td>
<td>Total/ Unique Clustered (No. of strains)</td>
<td>Total/ Unique Clustered (No. of strains)</td>
<td>DR</td>
<td>PGRS</td>
</tr>
<tr>
<td>A (1.5 kb)</td>
<td>171</td>
<td>62/39</td>
<td>23 (131)</td>
<td>27/15</td>
</tr>
<tr>
<td>B (1.3 kb)</td>
<td>3</td>
<td>3/3</td>
<td>0</td>
<td>2/1</td>
</tr>
<tr>
<td>C (1.0 kb)</td>
<td>9</td>
<td>9/9</td>
<td>0</td>
<td>3/1</td>
</tr>
<tr>
<td>D (4.5 kb)</td>
<td>3</td>
<td>3/3</td>
<td>0</td>
<td>2/1</td>
</tr>
<tr>
<td>Total</td>
<td>186</td>
<td>77/54</td>
<td>23 (131)</td>
<td>34/18</td>
</tr>
</tbody>
</table>

Table 14

IS6110 Single copy Cluster Analysis by Additional DR & PGRS RFLP
A big cluster (A-1.5 kb) of 171 strains was further differentiated into 62 DR and 27 PGRS patterns, of which 39 DR and 15 PGRS were unique. Thus, 36% of single copy A group strains got differentiated by additional DR and only 16% by PGRS, but with the combination of these probes, the differentiation was up to 50% (Table 14).

IS6110 RFLP patterns involved in clusters are shown in Figures 17a & 17b with the reference strain. A total of 24 patterns were involved in low copy clusters and 13 were involved in high copy clusters. Most of the clusters (23+11=34) were small with 2 to 3 isolates in each cluster with band frequency ranging from 2 to 15. The other 3 clusters (2H, 4B and 5F) consisted of 7, 9 and 4 isolates respectively. Overall, more clustering was observed in the low copy group. In all, 183 patients out of 451 (40%) were clustered in total 44 clusters when analysed by IS6110 & DR probes. With additional PGRS typing, the number of patients clustered was further reduced to 106 (23%). More number of patients (131) were clustered in IS6110 single copy group.
Conclusion
A combination of two to three genetic markers is able to differentiate the most endemic strains of *M. tuberculosis* in areas with a high incidence of tuberculosis.

Large restriction fragment polymorphism analysis of *M. chelonae* and *M. terrae* Isolates

Background
Assessment of genetic diversity is important in epidemiological studies of nontuberculous mycobacteria (NTM), as data from these studies could be used to monitor trends in the occurrence of new strains, identify possible sources of infection, and differentiate individual strains. In addition, polymorphism studies may have value in providing comparative information for the basis of human colonisation, infectivity, and virulence.

Aim
To examine the genetic relationships among several *M. chelonae* and *M. terrae* isolates obtained from different geographical sources for large-restriction-fragment (LRF) polymorphism by pulsed field gel electrophoresis (PFGE).

Methods
Six isolates of *M. chelonae* and four isolates of *M. terrae* obtained from different sources in this area were analysed by PFGE to examine LRF polymorphism using the chromosomal DNA digested with *DraI* and *XbaI* restriction enzymes. Species identification of *M. terrae* complex isolates was confirmed by mycolic acid analysis by high performance liquid chromatography. PFGE was performed with contour-clamped homogeneous electric field mapper system XA.

Results
PFGE pattern analysis was done by visual comparison of the number and similarity of bands. With the exception of one isolate of *M. terrae*, DNA from all other isolates could be digested with *DraI* and *XbaI* and resulted in separable fragments. Visual comparison of the LRFs showed a unique pattern for each of the isolates tested. A computer-assisted dendrogram of the percent similarity demonstrated a high degree of genetic diversity in this group of isolates. A summary of PFGE results for *M. chelonae* and *M. terrae* isolates are given in Table 15.

Conclusion
This study demonstrated that species of NTM, particularly *M. chelonae* and *M. terrae*, can be successfully typed by their LRF pattern using PFGE, which does not require species-specific DNA probes. The unique pattern obtained for each of the isolates demonstrated that there was a high degree of genetic polymorphism between the isolates originating from

Table 15

<table>
<thead>
<tr>
<th>Sample</th>
<th>Strain</th>
<th>Isolate source</th>
<th>Identification</th>
<th>No. of large restriction fragments</th>
<th>Genome size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A86/3</td>
<td>Soil</td>
<td>M. chelonae</td>
<td>10</td>
<td>4,050</td>
</tr>
<tr>
<td>2</td>
<td>D73/123</td>
<td>Soil</td>
<td>M. chelonae</td>
<td>11</td>
<td>4,410</td>
</tr>
<tr>
<td>3</td>
<td>B85/3ab</td>
<td>Water</td>
<td>M. chelonae</td>
<td>11</td>
<td>4,785</td>
</tr>
<tr>
<td>4</td>
<td>B86/12</td>
<td>Water</td>
<td>M. chelonae</td>
<td>11</td>
<td>4,160</td>
</tr>
<tr>
<td>5</td>
<td>TS09896</td>
<td>Sputum</td>
<td>M. chelonae</td>
<td>11</td>
<td>3,869</td>
</tr>
<tr>
<td>6</td>
<td>TS10108</td>
<td>Sputum</td>
<td>M. chelonae</td>
<td>10</td>
<td>4,090</td>
</tr>
<tr>
<td>7</td>
<td>TMC 1542</td>
<td>Water</td>
<td>M. chelonae</td>
<td>10</td>
<td>4,166</td>
</tr>
<tr>
<td>8</td>
<td>B92/1</td>
<td>Water</td>
<td>M. terrae complex</td>
<td>8</td>
<td>3,510</td>
</tr>
<tr>
<td>9</td>
<td>TS10088</td>
<td>Sputum</td>
<td>M. terrae complex</td>
<td>11</td>
<td>4,295</td>
</tr>
<tr>
<td>10</td>
<td>TS11431</td>
<td>Sputum</td>
<td>M. terrae complex</td>
<td>10</td>
<td>NPa</td>
</tr>
<tr>
<td>11</td>
<td>TS16563</td>
<td>Sputum</td>
<td>M. terrae complex</td>
<td>10</td>
<td>4,226</td>
</tr>
</tbody>
</table>

*M. chelonae* strain TMC 1542 was used as a reference strain.
Species identification by biochemical methods and high-performance liquid chromatography.
Approximate genome size (in kilobases) based on *DraI* digestion results.
NP, no pattern.
the same geographical area. Animal pathogenicity studies of *M. chelonae* and *M. terrae* may help in determining their difference in colonisation and pathogenicity.

**Studies in Progress**

**Identification of immunoreactive T cell antigens of *M. tuberculosis* through proteomic approach**
The major aim of this project is to identify a set of immunologically relevant T cell antigens and evaluate the response to these antigens in patients with tuberculosis and controls.

H37Rv culture filtrate and sonicate antigens have been prepared and separated using two-dimensional preparative electrophoresis, (First dimension Preparatory isoelectric focusing in fluid phase; Second dimension Preparatory SDS-PAGE by Whole gel eluter). Experiments are underway to identify the immunologically relevant T cell antigens by comparing the *in vitro* proliferative response and cytokine (IFN-γ) response in tuberculous and control subjects. The identified antigens will be characterised using proteomics approaches.

**Role of HLA-DR2 and mannose binding lectin (MBL) gene variants**
Earlier studies have revealed the association of HLA-DR2 and functional mutant homozygotes of MBL (MBL) gene variants with susceptibility to pulmonary tuberculosis. The present study has been planned to understand the immunoregulatory role of the polymorphic gene variants (HLA-DR2 and MBL) on the immune mechanism of tuberculosis susceptibility.

The study will be carried out in 60 pulmonary tuberculosis patients and 60 normal healthy volunteers. DNA typing of MBL genotypes, macrophage phagocytosis and lymphocyte response will be studied. Perforin positive cells will be enumerated.

During the year, 20 normal subjects and 20 pulmonary TB patients have been studied.

**Toll-like receptor expression profile in tuberculous pleuritis**
Toll-like receptors (TLRs) are pattern recognition receptors that mediate a link between the innate and adaptive immune response. When triggered, these receptors enhance the secretion of pro-inflammatory cytokines, which are imperative for resistance against tuberculosis. Of the nine receptors described for humans, TLR-2 and TLR-4 play a central role in immunity to tuberculosis. Study on the expression profile of TLRs in the cells obtained from the site of infection would provide insight into the role of these receptors *in vivo*. The expression profile of TLR-2 and TLR-4 in various immune cells like CD₄ T cells, CD₈ T cells, B-cells and monocytes obtained from both peripheral blood and pleural fluid are being analysed.

**Molecular and immunological characterisation of *M. tuberculosis* strains with single copy of IS6110**
Immune response induced by sonicate antigen (S7) in healthy subjects and TB patients
In an earlier study, it was shown that S7, one of the sonicate antigens from predominant clinical isolate, induced increased T cell proliferation with decreased IFN-γ secretion in healthy subjects, indicating diminished protective immune response. The aim is to understand the failure of this antigen to stimulate Th1 type of immunity and to evaluate its association with virulence. The study looks at the cytokine profile (Th1 / Th2 type) elicited by S7 sonicate antigen in comparison with H37Rv and PPD in healthy and pulmonary TB subjects. The cytokine profiles will be confirmed by intracellular staining for IFN-γ / CD₄ T cells and IL-4 / CD₄ T cells by FACS to dissect the type of immune response. The antibody, IgG and IgA levels in the plasma of these study subjects will also be evaluated using ELISA as an additive parameter for confirmation of the immune response.

**The innate immune response of polymorphonuclear leucocytes (PMN) in tuberculosis.**
PMN influx, the first line of defence, occurs as an early response against mycobacterial infection and plays a significant role in culminating the progression of infection. Chemokines stimulate the migration of PMN
from circulation to the site of infection and subsequently activate mycobactericidal function associated with the secretion of pro-inflammatory cytokines such as the TNF-α and IL-1β. These signals could either promote, or delay, the apoptosis of PMN. The study looks at the rate of PMN apoptosis induced by predominant strains of \textit{M. tuberculosis} from clinical isolates in correlation with intracellular survival of bacilli.

**Construction of recombinant BCG-based HIV-1 epitope delivery system**
The aim of this study is to construct a recombinant BCG based HIV-1 epitope delivery system. \textit{M. tuberculosis} Cpn10 antigen was used as a carrier and HIV-1 principal neutralising determinant (PND) epitope was used as a test epitope. Recombinant BCG expressing this chimeric antigen was constructed and animal experiments are being carried out to evaluate its immunogenicity.

**Serine threonine protein kinase, \textit{PknE} of \textit{M. tuberculosis}, H37Rv – cloning, expression and characterisation**
Serine threonine protein kinases of \textit{M. tuberculosis} are speculated to possess important physiological roles and are putative virulence factors. The principal aim of the project is to clone, express and purify the serine threonine protein kinase \textit{PknE}. Functional characterisation of the protein kinase is also being attempted by utilising a knockout mutant of \textit{pknE} in \textit{M. tuberculosis}.

Cloning, expression and purification of one of these kinases, \textit{PknE}, has been carried out. A targeted gene knockout of the corresponding gene was constructed in \textit{M. tuberculosis} by the specialised transduction strategy. Experiments are in progress to determine its biological function and role in the pathogenesis of tuberculosis.

**Functional genomics of mycobacterial genes involved in signal transduction**
The study aims to obtain information on the functions of those genes that are associated with the signal transduction machinery of \textit{M. tuberculosis} using genomic and proteomic approaches. A gene has been currently cloned and sequenced that encodes a penicillin-binding protein, and experiments are being carried out to overexpress and characterise the protein.

**Characterisation of DNA-binding factors regulating \textit{M. smegmatis} acetamidase operon**
The aim of this study is to characterise the transacting factors regulating \textit{M. smegmatis} acetamidase operon.
The mechanisms involved in the regulation of the highly inducible acetamidase gene of _M. smegmatis_ is also being studied. Work is currently underway to purify and characterise the regulatory proteins involved in the stringent control of the gene using a three-step chromatographic approach.

**Cloning genomic DNA fragments from _M. microti_ and screening for promoter activity using _E. coli-_mycobacteria shuttle plasmid vector**

Mycobacterial gene regulation and expression could be a lead in designing new recombinant DNA vaccines for tuberculosis and in finding various factors that control host specificity. Attempts are being made to screen inserts of _M. microti_, a member of _M. tuberculosis_ complex for promoter activity.

In this work genomic DNA fragments from _M. microti_ were digested and cloned in _E. coli-_mycobacteria promoter probe shuttle plasmid vector pJEM13 with _LacZ_ gene and an attempt was made to screen for promoter activity. _E. coli_ DH5 strain was used for the transformation. As a control, plasmid DNA of pJEM13 was used to check the transformation efficiency. Transformation was checked by the presence of kanamycin-resistant _E. coli_ cells. Repeated ligation and transformation did not yield blue colonies, indicating the absence of promoter activity. However, the appearance of white colonies indicated the expression of kanamycin-resistant genes. Of the white colonies six were selected for extraction of plasmid DNA and for the presence of inserts by standard procedures. Three plasmids (pAZM1-8 kb, pAZM2-4 kb and pAZM3-1.7 kb) with different size of inserts were obtained. The clone pAZM1 was large enough to carry more than one gene and it would be an interesting clone to study in detail.

**Analysis of molecular basis of fluoroquinolone resistance in mycobacteria**

Since fluoroquinolones are active against _M. tuberculosis_, they are being tried in combination with other anti-tuberculosis drugs in the treatment of TB. Due to their widespread usage in other bacterial infections, the emergence of resistance to quinolone has been recognised even before its use in the treatment of mycobacterial infections.

This study has been undertaken to amplify QRDR region using appropriate primers and sequencing for the detection of mutations, and to determine the level...
of resistance, by comparing the mutations of resistant strains with the susceptibility pattern of the same.

**Response of host signal transduction pathway to intracellular mycobacterial infection**

The broad objective is to study the host signal transduction pathways, especially the MAP kinase pathway, in response to intracellular *M. tuberculosis* infection. The present study mainly focuses on finding out the differential induction of the MAP kinases in the human macrophages and THP-1 cell line (human monocytic cell line), when challenged with a virulent clinical isolate and a low virulent strain of *M. tuberculosis*. Results of this study will shed light on the mechanisms of colonisation of mycobacteria in macrophages and the specific signalling pathway which it triggers in order to combat the host immune responses.

**Studies on complement – *M. tuberculosis* interaction**

Three intramural projects were initiated last year pertaining to a) levels of complement in tuberculosis, b) activation of complement system by genetically modified mycobacteria and c) the role of antibodies in the interaction between *M. tuberculosis* and macrophages through the complement system.

Since these are new projects, techniques pertaining to these are being standardised presently.

**Correlation between CD<sub>4</sub> counts, plasma viral load and IFN-γ levels in HIV-positive individuals, before and after completion of anti-tuberculosis treatment**

A study to correlate the stage of immunosuppression, viral load and cytokine levels in HIV-infected individuals is being carried out. Plasma viral load, CD<sub>4</sub> lymphocyte counts and IFN-γ levels will be estimated in the following groups of individuals:

- HIV-positive patients with tuberculosis;
- HIV-positive patients without tuberculosis;
- HIV-negative patients with tuberculosis; and
- Normal healthy controls.

These estimations will be undertaken on a second occasion in groups (a) and (c) at the end of anti-tuberculosis treatment. Viral load estimation will be carried out using a fully automated COBAS-AMPLICOR system. CD<sub>4</sub> lymphocyte counts will be performed by flow cytometry. The mononuclear cells will be stained with monoclonal antibodies against CD<sub>3</sub>, CD<sub>4</sub> and CD<sub>8</sub> cells. The fluorescent tags to be used are FITC, PE and PC5. It is planned to recruit 25 to 30 subjects in each group. Intake for the study is in progress.

**Cellular immune response in HIV-infected children with and without HAART**

This study is planned to assess the effect of HIV antigens/proteins to elicit an *in vitro* response in children with HIV infection. The results obtained from ELISpot will be compared with that obtained from intracellular cytokines.

About 25 to 30 HIV-infected children in the age group of 5-15 years will be recruited to the study, along with an equal number of normal healthy volunteers. The study population will be studied for the parameters of CD<sub>4</sub>/CD<sub>8</sub> counts, viral load, *in vitro* lymphoproliferative response, intracellular cytokine production and ELISpot analysis to detect IFN-γ production in response to various HIV and TB antigens/proteins. It is planned to execute the study in children before and after six months of treatment with antiretroviral drugs.
**Statistical Research**

**Studies Completed**

**Statistical modelling of HIV/AIDS epidemic**

AIDS is a devastating disease caused by HIV, which is transmitted by either sexual or other contacts in which body fluids are exchanged. The first case was reported among homosexual men in the USA in 1981. It has reached pandemic proportions, as no country in the world is free from HIV/AIDS. It ranks as one of the most destructive microbial scourges in human history and poses a formidable challenge to the biomedical research and public health communities of the world. AIDS and its related syndromes have changed virtually every aspect of medicine and society at large. The first case in India was reported in 1986. AIDS is a condition in which the inbuilt immune mechanism of the human body breaks down completely. The process is gradual but ultimately suppresses the immunity of the individuals.

The major modes of transmission are homo- or heterosexual contacts, transfusion of infected blood products, sharing of unsterile needles, and from the mother to the child, either during pregnancy, childbirth or during breast-feeding. Some of the important high-risk groups are people who have sex with multiple partners, commercial sex workers, truck drivers, homosexuals, STD clinic attendees, migrant workers, slum dwellers, street youths, blood recipients, and intra-venous drug users. The important approaches for modelling the spread of HIV/AIDS are deterministic, stochastic, state-space and statistical models.

**Back-calculation method**

This method reconstructs the past pattern of HIV infection and predicts the future number of AIDS cases with the present infection status. It depends on three important factors: incubation distribution, incidence curve and observed number of AIDS cases over time. This method is very popular and requires less information and assumptions. Lack of information about incubation distribution, the effect of intervention therapy on incubation period, and errors in reported AIDS incidence leads to uncertainties associated with this method.

HIV incubation period is the random time between the HIV-infection and the onset of clinical AIDS. Distribution of this non-negative random variable is known as HIV incubation period distribution. It is assumed to be exactly known in back-calculation methodology. Incubation period of HIV is very long and highly variable within and between cohorts. The current prevalence of HIV-infection and the corresponding pattern of incidence from the beginning of the epidemic to the present time are mainly estimated by means of back-calculation method. It calculates the most likely temporal distribution of infected individuals compatible with the number of observed AIDS cases starting from the suitable estimate of the incubation period, derived from the available data. Most of the projections formulated the problem of estimation of future AIDS cases as estimation of parameters in multinomial likelihood with unknown sample size by EM algorithm. The most commonly used infection curves in back-calculation for incidence and prevalence are linear, quadratic, exponential, root exponential, double exponential, logistic and log-logistic.
The back-calculation method uncertainties are due to incubation period distribution, infection density and AIDS incidence data. Simulation techniques were used to examine the variation associated with the back-calculation estimates of HIV/AIDS and to quantify the extent of uncertainties due to variation in the incubation distributions and infection curves. Assuming a multinominal distribution with unknown sample size for the reported AIDS cases, the Indian surveillance databases were simulated. NACO reported around 4 million people living with HIV infection by the end of 2002. For the simulation, we have taken 4 million as the prevalence to simulate the AIDS cases. The incubation period was assumed to be Weibull model with median 10 and 15 years.

In order to quantify the effects of incorrect specification of incubation period distributions on the back-calculation estimates, seven most commonly used models, namely gamma, log-logistic, log-normal, generalised exponential, generalised log-logistic, generalised gamma, mixed Weibull and two newly proposed models, namely change point model and immune invasion model, were fitted to 5000 observations simulated from Weibull model. The simulations and comparisons were done using SAS software. Parameter estimates of alternative models when the true, underlying incubation period distribution is Weibull, were studied.

The results indicated that log-logistic and root exponential models gave expected AIDS cases significantly different from the simulated results. The minimum size of the epidemic, estimated by exponential and double exponential methods, was high compared to other curves. Logistic incidence, exponential and double exponential gave consistent estimates for projection of AIDS. Assuming a median of 10 years for data generated, the changes in the incubation period were very much affected by the minimum size of the epidemic. The coefficient of variation was in the order of 18% under the true model accounting for variations in the incubation period, which rose up to 65% if the infection densities were also varied. Total epidemic size was affected by the changes in the incubation period.

The projected estimates were similar to changes in the incubation period. The simulation study further revealed that the infection density had to be chosen judiciously. The estimates are affected by improper choice of the infection densities. The same pattern of uncertainty is reflected for median incubation period with 15 years.

Estimation of parameters

The starting year of the infection for India was taken as 1981. The projection of AIDS estimates were obtained for four median periods, namely 8, 10, 12 and 15 years. Prior estimates of the parameters for the incubation models – Weibull, gamma, log-logistic, lognormal and generalised exponential – are available. For these models one parameter was fixed based on the prior estimates and the other was calculated with median distribution periods of 8, 10, 12 and 15 years. Parameter estimates of the other models, namely generalized log-logistic, generalised gamma, mixed Weibull, change point and immune invasion, are not available. The estimates were decided from the simulation study. The infection densities used were logistic, log-logistic, exponential, root exponential and double exponential.

HIV/AIDS estimates for India

Back-calculation estimates were obtained using the conditional likelihood approach for the multinominal distribution with unknown sample size. Non-linear optimisation routines were used for maximisation of the conditional likelihood. The estimates of the minimum number of AIDS cases, HIV incidence and short-term projection were obtained. The important findings are:

a) Projected AIDS cases do not vary much across various infection densities and incubation period distributions.

b) Minimum size of the epidemic and HIV incidence vary across the infection densities and incubation distributions.

c) The projected AIDS cases using the log-logistic and root exponential infection densities are less as compared to the others.
d) The projected models-based median AIDS cases in India for the years 2003, 2004, 2005 and 2006 are around 27,000, 44,000, 70,000 and 114,000 respectively.

Adjustments for errors in reporting
The level of under-reporting in the South-East Asian countries, including India, as reported by WHO was around 80% in 1993. The reported AIDS cases in India were adjusted upwards, assuming 90% under-reporting during 1986-87 and decreasing to 50% in 2001-02, in an exponential decay form. The over-dispersed Poisson regression model with modifications, as adopted by Vandal and Remis for Canadian projections, was used for adjusting the reporting delays. The revised AIDS estimates after adjustments for 2003, 2004, 2005 and 2006 are around 67,000, 100,000, 153,000 and 230,000 respectively.

Studies in Progress

Evaluation of cost-effective biomarkers for HIV staging and monitoring
The analysis of marker data from HIV-positive patients is one of the important topics in HIV/AIDS research. Many markers have been suggested as indicators of the progression of HIV disease. The markers that have been most frequently used are the $CD_4$+ T-lymphocyte counts ($CD_4$) and HIV viral load (VL). Both markers have been shown independently predictive of disease progression, but are very expensive measurements. There also exist differences between laboratory methods in estimating the markers. It is known that the normal ranges of $CD_4$ and CD8 lymphocyte counts vary significantly among different ethnic groups, and Caucasians have higher $CD_4$ counts than Asians. Other important markers considered are total lymphocyte counts (TLC), haemoglobin and others, and some of them have been shown to have relationship with disease progression. But none of them have been established, either as a clinical tool for evaluating patients end points, or as a surrogate end point in therapeutic trials.

There is a need for alternative and less expensive markers, and an alternate to $CD_4$ for staging readiness for treatment and monitoring response to treatment. There is also a need to calibrate the strategy according to the relative costs of false positive and false negative results, and the proportion of the population that needs to be treated. Considerable recent activity has focused
on the use of TLC as a surrogate marker for CD₄. For HIV staging, some of the researchers reported TLC as a good marker for predicting CD₄ as well as for staging and monitoring. However, there are also others who contradicted reports on TLC as a good marker.

The data from our Centre shows that TLC is an imperfect predictor of CD₄. It also varies considerably between populations. For example, the West Africans are reported to have about 30% higher TLC than Europeans for the same CD₄. The CD₄% and TLC vary considerably within population. For example, the Centre’s clinical trial patients’ CD₄ % varied from 4 to 48% and the TLC varied from 200 to 9600. The ROC curve has its limitations in devising optimal cut-off points in these situations. The decision theory approach using clinical utility functions will be a better approach and this work discusses this methodology.

Our focus is on staging (CD₄ < 200 and CD₄ ≥ 200). To evaluate predictive accuracy and to develop diagnostic rules based on cut points, the decision theory approach is used. The decision rule is: observe TLC < k assume CD₄ < 200. Otherwise assume CD₄ ≥ 200. The sensitivity is the fraction of those with TLC < k among those with CD₄ < 200. That is: SE (k) = pr (TLC < k | CD₄ < 200).

The specificity is the fraction of those with TLC > k among those with CD₄ ≥ 200. That is SP (k) = pr (TLC > k | CD₄ ≥ 200). The prevalence is the fraction of the population with CD₄ < 200 π = Pr (CD₄ UC < 200).

**Loss function approach**

If the decision rule is to treat if TLC < k, the loss function is constructed by adding up proportion of false positives and false negatives that result from using this cut point L (K) = pr(FP | k) + pr(FN | k). The loss function is a function of sensitivity, specificity, and prevalence. Rewrite loss function L(k) = FNR(k) π + FPR(k) (1 − π).

The implication is that the optimal cut-off point depends on the true proportion that needs treatment (π). Hence optimal treatment strategy depends on the population where treatment is being administered. If π is near 1.0 minimise false negatives (treat everyone), and if π is near 0 minimise false positives.

If false negative is more costly than false positive, the weights can be appropriately assigned in the construction of loss function. The optimal cut-off point k depends on the relative costs of wrong decisions. Optimising the value of k by α and π are carried out. Work is currently under progress to incorporate generalised utility functions to the loss function and to use change in TLC to monitor treatment effects.
Information Services

Library

The TRC has an excellent library, having print and digital resources. It has made productive and commendable progress in online resources and services.

Renovated library

The library was renovated with the establishment of an Internet Browsing Centre. Internet connectivity has been established with DSL high bandwidth. The modular library provides beautiful learning ambience.

Digital library

The TRC Digital Library was established and is serving as a Web-based interface to the in-house resources and hyperlink to all the electronic journals subscribed by the Centre. It facilitates the staff of the Centre to access it as a one-stop-shop for electronic resources through Local Area Network at their desk. The digital library has been strengthened substantially with full-text e-journals; e-databases; e-subject collection and publisher’s cumulative collection.

The library services are laid down to gather health information and make it accessible to the scientific community. We also provide pointers to outside resources through search engines.
and health science databases through digital library process, since TRC does not possess repository for these.

The institute retained its institutional membership facility at the British Council Library, Chennai. The existing resource sharing facility with the National Institute of Epidemiology has further been elaborated with online databases through ICMR consortium database subscription for southern region institutes. The library continues to act as a central node for electronic mail co-ordination for the staff of the Centre.

The electronic collection development during the year is indicated below:

1. American Society for Microbiology (Pub. Cumulative collection)
2. Annual Reviews: Biomedical Suite (Since Vol.1+)
3. ELSEVIER
   a. BioMedNet Reviews
   b. Science Direct
      b.1 Immunology & Microbiology (Sub. Collection)
      b.2 Individual titles-5
4. Online journal subscription - 11

Electronic Data Processing

The electronic data processing division is continuing to give data management support, including data entry/verification to various studies undertaken at the Centre. Apart from data processing and computer programming, this division is taking care of all the PCs and printers by bringing under comprehensive maintenance contract service to avoid breakdown. Also, annual procurement of computer consumables is done by making indent through this division for use in various departments of TRC.

The routine data outputs are being supplied for monitoring the studies and carrying out the field work on schedule. The quantum of documents entered and verified from April 2003 to March 2004 is shown below:

No. of documents entered : 1,88,234
No. of documents verified : 1,84,678

Apart from regular data management, this division assists in analysing the data for research work and publication. Also, it helps in preparation of employees’ pay roll, income-tax sheets, loan schedules and central bills.

The LAN computer system in the division is catering its service continuously, mainly to access information through Internet facility and to communicate among staff through intranet facility.
Publications

Published in

(i) International journals: 23
(ii) National journals: 19

Accepted for publication in

(i) International journals: 17
(ii) National journals: 9

Published International

1. Priya Rajavelu and Sulochana Das. Cell-mediated immune responses of healthy laboratory volunteers to sonicate antigens prepared from the most prevalent strains of *Mycobacterium tuberculosis* from south India harbouring a single copy of IS6110. *Clinical and Diagnostic Laboratory Immunology, 2003, 10, 1149–1152.*


National


7. Tuberculosis Research Centre. Operational research studies in tuberculosis (TB) control – Contributions from Tuberculosis Research Centre. Prepared by Dr. Rani Balasubramanian. In the souvenir released at the 54th TB seal campaign 2003 entitled “TB seals on Historical Monuments” by The TB Association of India, New Delhi.


**Accepted for publication**

**International**

1. B. Ramalingam, Alain R. Baulard, Camille Locht, P.R. Narayanan, Alamelu Raja. Cloning, expression and purification of the 27 kDa (MPT51, Rv3803c) protein of *Mycobacterium tuberculosis*. *Protein Expression and Purification*.


centres. *International Journal of Tuberculosis and Lung Disease.*

10. N. Selvakumar, K.J. Ilampuranan, C. Ponnuraja, P.R. Narayanan. Increased detection of acid-fast bacilli in sputum samples preserved in cetylpyridinium chloride solution by a modified Auramine-Phenol staining procedure. *Journal of Clinical Microbiology.*

11. N. Selvakumar, S. Sivagamasundari, E. Prabhakaran, R. Govindaraju, M. Perumal, Rueben Granich, Fraser Wares, L.S. Chauhan, P.R. Narayanan. Storage of heat-fixed unstained sputum AFB smears for panel testing in a Tuberculosis Unit in south India. *International Journal of Tuberculosis and Lung Disease.*


13. G. Ramachandran, E.S. Perloff, L. von Moltke, S. Swaminathan, D.J. Greenblatt. Analysis of generic antiretroviral medications in India. *AIDS.*


### National


4. Tuberculosis Research Centre. Operational research studies in TB control: Contributions from Tuberculosis Research Centre in the last two decades. *SAARC Journal of Tuberculosis, HIV/AIDS and Lung Diseases.*


7. N. Selvakumar, Vanaja Kumar, P.G. Gopi, S. Sivagamasundari, E. Prabhakaran, Samuel
Vasanthan, M. Perumal, P.R. Narayanan. Sputum AFB smear reading capability of Senior Tuberculosis Laboratory Supervisor trainees under training at a reference laboratory in India. *Indian Journal of Tuberculosis.*


Others

1. Rani Balasubramanian, Rajeswari Ramachandran. “Evolution of chemotherapeutic regimens in the treatment of tuberculosis and their scientific rationale” chapter for a book on “Tuberculosis” published by Dr. S.K. Sharma, Professor of Medicine, All India Institute of Medical Sciences, New Delhi, and Dr. Alladi Mohan, Professor of Acute Care Medicine, University of Thirupathy.

Awards

**Dr. Ranjani Ramachandran**

The paper entitled ‘Mycobacteremia in TB patients with HIV-infection”, which was presented at the 57th National Conference on Tuberculosis and Chest Diseases, held at Goa during October 2003 was awarded Prof.K.C. Mohanty Award for the best paper

**Ms. Radha Gopalswami**

The paper titled “Cloning, expression and characterization of serine threonine kinase *pkn* of *M.tuberculosis*” bagged the Best Poster Award at the 30th Annual Conference of Indian Immunology Society held at Sanjay Gandhi Postgraduate Institute of Medical Science, Lucknow, during November 2003.

**Dr. Geetha Ramachandran**

The paper titled “Vitamin A levels in sputum positive pulmonary tuberculosis patients in comparison with household contacts and healthy normals”, which was presented at the 58th National Conference on Tuberculosis and Chest Diseases held at Mumbai during January 2004, has won the Dr.C. Srinivasa Rao Award for the best paper on tuberculosis for the year 2003.

**Dr. S. Ramesh Kumar**

The paper titled “Impact of HIV-infection on chest radiographic findings in patients with pulmonary tuberculosis”, which was presented at the National Conference on Management of HIV/AIDS in Resource Restricted Settings at Mumbai during February 2004, bagged the second prize for the best poster presentation.

Advocacy

**Dr. Aleyamma Thomas**

*Facilitator for :*

Training programme for Deputy Directors, Health Services of Tamil Nadu state during May 2003.

RNTCP training for Medical Officers at Andaman & Nicobar Islands during Nov. 2003.

WHO trainees from DPR Korea during Apr. 2003.


**Dr. Pauline Joseph**

*Facilitator for :*

Training programme for Deputy Directors, Health Services of Tamil Nadu state during May 2003.

RNTCP training for Medical Officers at Andaman & Nicobar Islands during Nov. 2003.

WHO trainees from DPR Korea during Apr. 2003.


Dr. Rani Balasubramanian
Facilitator for training programme for Deputy Directors, Health Services of Tamil Nadu state during May 2003.

Delivered a lecture on “Public-private Models in RNTCP and the role of Medical colleges” to Medical Officers in RNTCP at Chennai on 11th Dec. 2003.

Delivered a lecture on “Scientific basis of DOTS strategy” and “Management of extra-pulmonary forms of TB” to Medical Officer trainers from Bangladesh at Chennai during Dec. 2003.

Facilitator for training programme to field investigators and medical social workers enrolled for the multicentric study entitled “Timing and its significance in the diagnosis and treatment for TB” and organized focus group discussions among laboratory technicians and community DOT providers.

Biochemistry Department
Training on HPLC methodology and anti-TB drug assays to undergraduate, postgraduate and M.Phil. students of neighbouring educational institutions.

Bacteriology Department
Training in Drug Resistance Surveillance provided to staff members of CJIL, Agra, Malankara Orthodox Syrian Church Medical College, Kolenchery, Kerala and Institute of Medical Science, Kalapet, Pondicherry.

Statistics Department
Bio-statistical consultation offered to:
- Medical Officer from Sundaram Medical Foundation, Chennai.
- Postgraduate and M.Phil. students from Loyola College, Chennai.
- Postgraduate students from Presidency College, Chennai.
- M.D. students from Stanley Medical College, Chennai.
- Medical Officer, Dept. of Paediatrics, Southern Railways, Chennai.

Pathology Department
Summer training for postgraduate (Biochemistry & Microbiology) and B.Tech. (Biotechnology) students during Apr. – June 2003.

HIV/AIDS Division
Training provided for students from the Department of Social Work of Madras Christian College, Loyola College and Madras School of Social Work, Chennai.

Capacity Building

Staff and students who were awarded their Ph.D. degree during the year:
1. Dr. S. Selvakumar - The Tamil Nadu Dr. M.G.R. Medical University – (Guide - Dr. Sujatha Narayanan)
2. Dr. P. Vijayalakshmi - Madras University – (Guide - Dr. P.R. Narayanan)
3. Dr. Daisy Vanitha - The Tamil Nadu Dr. M.G.R. Medical University – (Guide - Dr. C.N. Paramasivan)
4. Dr. S. Anitha – Madras University (Guide – Dr. P. Venkatesan)
5. Dr. V. Kamalakannan - Madras University – (Guide - Dr.P.R. Narayanan)
6. Dr. B. Ramalingam -The Tamil Nadu Dr.M.G.R. Medical University – (Guide-Dr. Alamelu Raja)

Dr. Ranjani Ramachandran and Ms. Sulochana Somasundaram
Training on laboratory procedures for the diagnosis of SAARS-associated Corona virus by PCR at National Institute of Virology, Pune, during May 2003.
Dr. Geetha Ramachandran  
Training in pharmacokinetics of antiretroviral drugs at Tufts University School of Medicine, Boston, USA, during Aug. – Dec. 2003.

Dr. P. Venkatesan  
Online Bioinformatics Course conducted by Life Science Informatics Alliance, Brown University, Providence, USA during Aug. – Nov. 2003.


One-semester course at Brown University, Providence, USA, in the following aspects:

- Computational Molecular Biology (Dept. of Applied Mathematics)
- Machine Learning (Dept. of Computer Science)
- Geographical Information System (Dept. of Population Sciences)
- Biomedical Ethics (Center for AIDS Research)

Dr. Luke Elizabeth Hanna  
Post-doctoral fellowship through the AIDS International Training Programme at Albert Einstein College of Medicine, New York, for 2 years from January 2004.

Dr. Pradeep Aravindan Menon  

Ms. Mohanarani Suhadev  

Special Assignments

Dr. Geetharamani Shanmugam  

Honorary member of the “Child Welfare Committee”, vested with a power of a District Magistrate, for Chennai district by the Department of Social Welfare, Govt. of Tamil Nadu for a period of three years from Jan. 2004.

Dr. M. Kannapiran  

Dr. C.N. Paramasivan  
Temporary Advisor, WHO, during Nov. 2003 at Bali, Indonesia (Meeting of the SEAR Technical working group on TB & meeting of the National TB Programme Managers of SEAR).

Nominated by the Govt. of India to participate in the workshop on lab management at SAARC TB Centre for a period of two weeks during Dec. 2003 at Kathmandu, Nepal.


**Dr. Prema Gurumurthy**
External examiner to conduct public viva-voce examination for Ph.D. students of the Tamil Nadu Dr. MGR Medical University, Chennai.

Member of Selection Committee of the Tamil Nadu Dr. MGR Medical University for Ph.D. candidates.

**Dr. Rajeswari Ramachandran**


**Dr. V.D. Ramanathan**
Consultant for the ILEP Nerve Function Impairement and Reaction in Leprosy Project.

Organizer of a workshop on ‘Confocal Imaging Techniques’ held at the Tuberculosis Research Centre, Chennai during Apr. 2003.

**Dr. Rani Balasubramanian**

Member of National Task Force constituted for involving all the medical colleges in the country in RNTCP.

**Dr. N. Selvakumar**
Member of Scientific Advisory Committee of CLTRI, Chengleput.

Consultant for External Quality Assurance in sputum microscopy at WHO-RNTCP consultants’ review meeting held at Jaipur during June 2003.

**Ms. Sudha Ganapathy**

**Dr. Sujatha Narayanan**
Examiner for Ph.D. thesis submitted to the University of Delhi.

Outside expert to review the work of autonomy in the Department of Biochemistry, Meenakshi College for Women (Autonomous), Chennai, Jan. 2004.
# Ph.D Scholars - Students

Students who have registered (full-time) for their Ph.D. programme at the University of Madras and working at Tuberculosis Research Centre

<table>
<thead>
<tr>
<th>Name</th>
<th>Source of Funding</th>
<th>Title</th>
<th>Supervisor-Guide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ms.R. Priya</td>
<td>ICMR</td>
<td>Apoptosis of human monocytes and macrophages by <em>M. tuberculosis</em> and its implications on cell mediated immune response</td>
<td>Dr. Sulochana Das</td>
</tr>
<tr>
<td>Ms. Nisha Rajeswari</td>
<td>ICMR</td>
<td>Influence of HLA-DR antigens on macrophage phagocytosis, perforin positive cells and cytokine response in pulmonary tuberculosis</td>
<td>Dr.P. Selvaraj</td>
</tr>
<tr>
<td>Ms.C. Prabha</td>
<td>ICMR</td>
<td>Immune response in tuberculosis: TH1/TH2 paradigm</td>
<td>Dr. Sulochana Das</td>
</tr>
<tr>
<td>Ms.M. Vidya Rani</td>
<td>ICMR</td>
<td>Regulatory role of variant genotypes of vitamin D receptor, interferon- and interleukin-4 genes on vitamin D₃ modulated immune response in pulmonary tuberculosis</td>
<td>Dr.P. Selvaraj</td>
</tr>
<tr>
<td>Ms.G. Shenbagavalli</td>
<td>ICMR</td>
<td>Serum and tissue complement profile in tuberculosis</td>
<td>Dr.V.D. Ramanathan</td>
</tr>
<tr>
<td>Ms.G. Radha</td>
<td>CSIR</td>
<td>Cloning, expression and characterization of a serine threonine protein kinase, <em>PknI</em> of <em>M. tuberculosis</em> H37Rv</td>
<td>Dr. Sujatha Narayanan</td>
</tr>
<tr>
<td>Mr.V. Aravindhan</td>
<td>CSIR</td>
<td>Construction of recombinant BCG based HIV-1 PND epitope delivery system</td>
<td>Dr. P.R. Narayanan</td>
</tr>
<tr>
<td>Mr. Deepak Jayakumar</td>
<td>CSIR</td>
<td>Cloning, expression and characterization of a serine threonine protein kinase, <em>PknE</em> of <em>M. tuberculosis</em> H37Rv</td>
<td>Dr. Sujatha Narayanan</td>
</tr>
<tr>
<td>Name</td>
<td>Source of Funding</td>
<td>Title</td>
<td>Supervisor-Guide</td>
</tr>
<tr>
<td>--------------------</td>
<td>-------------------</td>
<td>----------------------------------------------------------------------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td>Dr.P.L. Natarajan</td>
<td>CSIR</td>
<td>Cellular immunology of TB and HIV/TB</td>
<td>Dr. Sujatha Narayanan</td>
</tr>
<tr>
<td>Mr. D. Anbarasu</td>
<td>CSIR</td>
<td>Identification and characterization of immuno reactive T-cell antigens of <em>M. tuberculosis</em></td>
<td>Dr. Alamelu Raja</td>
</tr>
<tr>
<td>Mr. S. Manivannan</td>
<td>CSIR</td>
<td>The role of complement activation and antibody in the early interaction of <em>M. tuberculosis</em> and macrophages</td>
<td>Dr. V.D. Ramanathan</td>
</tr>
<tr>
<td>Mr. V. Narayana Rao</td>
<td>CSIR</td>
<td>Complement activation by strains of mycobacteria wild type and gene disrupted <em>M. tuberculosis</em> and recombinant BCG</td>
<td>Dr. V.D. Ramanathan</td>
</tr>
<tr>
<td>Ms. G. Chandra</td>
<td>UGC</td>
<td>Studies on the influence of vitamin D₃ and the polymorphic variants of vitamin D receptor gene on the immune functions to <em>M. tuberculosis</em> antigens in pulmonary tuberculosis</td>
<td>Dr. P. Selvaraj</td>
</tr>
</tbody>
</table>

Thirteen Ph.D. students along with the Director, TRC, visited Vector Control Research Centre, Pondicherry, and made presentations of their research activities in December 2003.
### Staff who have registered (part-time) for their Ph.D. programme at University of Madras, Chennai

<table>
<thead>
<tr>
<th>Name</th>
<th>Title</th>
<th>Supervisor-Guide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mr. R. Selvaraj</td>
<td>Statistical considerations in modelling <em>M. tuberculosis</em> infection with neural network</td>
<td>Dr. G. Gopal</td>
</tr>
<tr>
<td>Ms. Beena E. Thomas</td>
<td>Psychosocial and sexual impact of HIV/AIDS-Gender differentials</td>
<td>Dr. Shanmugavelayudam</td>
</tr>
<tr>
<td>Ms. Sulochana Somasundaram</td>
<td><em>In vitro</em> studies of quinolones against <em>M. tuberculosis</em></td>
<td>Dr. C.N. Paramasivan</td>
</tr>
<tr>
<td>Mr. L. Prabhakaran</td>
<td>Isolation, characterization and construction of luciferase reporter phage for diagnosis of <em>M. tuberculosis</em></td>
<td>Dr. P.R. Narayanan</td>
</tr>
<tr>
<td>Ms. Gomathy Sekar</td>
<td>Optimizing sputum microscopy to detect AFB</td>
<td>Dr. N. Selvakumar</td>
</tr>
<tr>
<td>Ms. N.S. Gomathy</td>
<td>Development of rapid methods for diagnosis and drug susceptibility testing of <em>M. tuberculosis</em></td>
<td>Dr. Vanaja Kumar</td>
</tr>
<tr>
<td>Ms. Nalini Sundar Mohan</td>
<td>Measurement of drug resistance in tuberculosis</td>
<td>Dr. C.N. Paramasivan</td>
</tr>
<tr>
<td>Ms. Mohanarani Suhadev</td>
<td>Sociological aspects of HIV/AIDS</td>
<td>Dr. Udaya Mahadevan</td>
</tr>
<tr>
<td>Ms. Silambu Chelvi</td>
<td>Antimycobacterial bioactive compounds from marine actinomycetes</td>
<td>Dr. Vanaja Kumar</td>
</tr>
<tr>
<td>Mr. C. Ponnuraja</td>
<td>Frailty models</td>
<td>Dr. P. Venkatesan</td>
</tr>
<tr>
<td>Mr. N. Arunkumar</td>
<td>Causal inference</td>
<td>Dr. P. Venkatesan</td>
</tr>
<tr>
<td>*Mr. M. Muniyandi</td>
<td>Economic and health status of tuberculosis patients covered under DOTS and non-DOTS programme in south India</td>
<td>Prof. G. Rama Rao</td>
</tr>
</tbody>
</table>

*Registered at International Institute of Population Sciences, Mumbai
For complete staff-list refer to www.trc-chennai.org