TUBERCULOSIS RESEARCH CENTRE

Chennai

Annual Report

January 2001 - March 2002

Tuberculosis Research Centre

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Preface

The road ahead.....

There is no reason why so many should die of tuberculosis or live with the disease when we know the blue print of the bacterium; diagnosis, in the majority of cases, is relatively easy and cheap; an array of powerful drugs are available to ensure cure, if properly used; and control programmes are in place since decades.

However, the enemy *Mycobacterium tuberculosis,* with its tough exterior and bag of biological tricks, outsmarts chemotherapeutic and diagnostic efforts. HIV and TB together are seriously threatening the best control programmes while undermining the efforts of lesser ones. To top this, population explosion, overcrowding and poverty help breed TB. Therefore, control programmes need to fight a continuous battle to curb the spread of the disease.

TB control programmes need better diagnostic tools to help detect the disease early and determine its spread in the community. They need better drugs to combat resistance and the "double-trouble" associated with HIV-TB in addition to shorter treatment regimens to ensure better compliance. A replacement for the BCG vaccine that offers unquestionable protection will be helpful. Finally they need practical solutions for the multitude of problems that crop up in field operations.

No control programme needs this as urgently as India's Revised National Tuberculosis Control Programme (RNTCP). Not only is it the world's most rapidly growing TB control programme, but also the most ambitious. It has problems in logistics and quality control as other programmes. In addition are added concerns such as the large numbers of patients to be identified and treated, capacity building and establishment of effective monitoring and surveillance networks. All of these require effective and sustainable strategies that can only come from research.

Tuberculosis Research Centre has a unique role to play in contributing to tuberculosis research. A quarter of a century ago it was a centre for controlled clinical and field trials in tuberculosis. Today, we have consolidated our position as one of the world leaders in the chemotherapy of tuberculosis. TRC's heritage and its comparative advantages allow it to excel in the fields of controlled clinical trials, mycobacteriology and epidemiology. Strategic planning and development of infrastructure have helped it to attain a position of strength in the area of modern biology. It is in the forefront of training and dissemination of information relevant to control programmes. TRC has the unique advantage of housing basic scientists working closely with clinicians and field workers, who ultimately apply the basic research findings to control TB in the community.

January 2001 to March 2002 were one of TRC's best years. We continued to build on our strengths by undertaking controlled clinical trials to explore shorter treatment regimens. We addressed key questions in the epidemiology of tuberculosis, such as assessing the annual risk of infection and the epidemiological impact of directly observed treatment, short course (DOTS). Additionally, we took on newer responsibilities in operational research and capacity building for the TB control programme. We rapidly expanded our portfolio to establish ourselves as a significant player in molecular epidemiology, genomics and socio-economic aspects of TB research. The centre continued its effort to focus on research in evaluating the diagnostic criteria and developing strategies for the prevention and treatment of TB in HIV infected persons.

We redesigned the way we build partnerships at all levels to promote development of new diagnostics, drugs and vaccines. Our model for private practitioner participation in tuberculosis control developed with ACT (Advocacy for Control of Tuberculosis) is one such example.

Tuberculosis remains neglected by policy makers, health administrators and the community. We changed the way we communicate with the TB and non-TB communities. To enhance advocacy, we disseminated several key messages about the disease, the diagnostic and chemotherapy tools available and effective ways of using them. Control programmes and health administrators, who were quick to recognise the value of TRC's endorsement of their campaigns, routinely seek our support. Our best platform for advocacy is our web site, *trc-chennai.org* that has received over 40,000 visitors during the last 2 years. We have facilitated links between public health, operational research and clinical care using our web site.

We recognise that our continued success is far from guaranteed unless we use newly available opportunities to build a holistic approach towards TB research. We are conscious that TB control and research are inextricably linked and research should feed the needs of control programmes. While we need to identify chinks in *M. tuberculosis'* armour to design better drugs and vaccines, we also need operational research to transfer the technology from the laboratory to the field.

Recognising the current needs of TB research and reminding ourselves of our goal of reducing morbidity and mortality due to TB, we have successfully reoriented our institute to address key issues related to health policy, systems and services research. The next steps for TRC should be to continue exploring gaps in current knowledge for TB control, to excel in laboratory based and operational research and to disseminate its findings to programme managers and policy makers. Through this we believe that TRC will effectively shift the focus of TB research in India.

Development of strategic plans for effective utilisation of funds, identifying time defined deliverables and building sustainable partnerships will be the backbone of the centre's approach towards achievement of this goal.

P.R. Narayanan

Director
prnarayanan@vsnl.com

Tuberculosis Research Centre

2001-2002

Contents

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Members

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New Delhi

Dr. G.R. Khatri
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Special Invitees

Dr. M. Palaniappan
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Chennai

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Dr. P. Krishnamurthy
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"…TRC is doing very good work both in research and training, and should assist more states to build capacity in TB research and control.."

Padmashri Dr. C.P. Thakur
H'ble Minister of Health &
Family Welfare
Govt. of India

"…TRC is in the forefront of the battle against the scourge of TB. The range of research covering vital operational aspects is really impressive. We in the Government of India are proud of this institution..."

Shri. J.A. Chowdhury
Union Health Secretary
Govt. of India

"…Finally got to visit one of the finest centres in TB research…"

Dr. Sandip K. Basu
Director
National Institute of
Immunology
New Delhi

"…a fascinating visit - concentrating on some wonderful work..."

Prof. Nicky Padayachee
Prof. of Public Health
Executive Dean
Faculty of Health Sciences
University of Cape Town
South Africa

"…TRC has revitalized and reoriented to do very useful and practical research of immediate value for India and the world..."

Dr. Fabio Luelmo
Medical Officer
STOP TB
WHO, Geneva

"…So much has happened over the years with great improvement in facilities and hopes for the future…"

Prof. D.A. Mitchison
St. George Hospital
Medical School
London, UK

"…Impressed with the dedication of all who I met…"

Dr. Karyl S Barron
Deputy Director
Division of International
Research
National Institute of Allergy &
Infectious Diseases
NIH, USA

"…A dedicated and dynamic group working at TRC and looking forward to future interactions…"

Dr. Stephaine Lynn James
Parasitology & International
Programs
National Institute of Allergy &
Infectious Diseases
NIH, USA

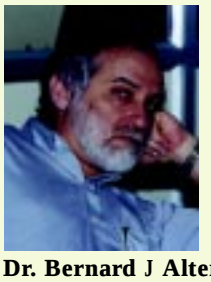
"…Facilities are good, the working environment quite congenial and the institute is doing a tremendous job in its area of operation…"

Mr. Madhavan
Health Secretary
Govt. of Haryana

"…Carrying out pioneering work in the area of TB…"

Dr. Pedro Medrano
Regional Manager
South Asia
WFP Representative
& Country Director
India

Dr. Marco Di Capua
Embassy of USA
Counselor for Science & Technology
Environment & Health Affairs
New Delhi



Dr. Bernard J Alter
Consul General of USA
Chennai

Dr. Thomas Nutman
National Institute of Allergy & Infectious Diseases
NIH, USA

Padmashri
Dr. (Mrs.) Shanthi Ranganathan
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TT Ranganathan Clinical Research Foundation
Chennai

Dr. Padmini Salgame
Associate Professor
Dept. of Microbiology & Immunology
Temple University
Philadelphia, USA

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Dr. Klaus Jochem
Health Canada
Canada Regional TB & HIV/AIDS

Dr. Prahalad Kumar, DD
SAARC TB Centre
Kathmandu, Nepal

Prof. Roger Detels
UCLA School of Public Health, Los Angeles
California

Workshops

- ICMR/WHO Workshop on "Ethical Issues in Biomedical Research"- 22-24 Jan. 2001
- Workshop on "Sensitization of District Magistrates/ Municipal Commissioners in RNTCP" -13 Feb. 2001
- WHO Workshop on "Research Dissemination" - 16-17 Mar. 2001
- NGOs Workshop on "Challenges in Mobilizing Community Support in HIV and TB Prevention and Control" organised by TRC/TNSACS - 17 Apr. 2001
- WHO Workshop on "Laboratory Based Disease Surveillance" - 23-25 May 2001
- ICMR/WHO Workshop on "Use of Information Technology (IT) in Biomedical Research" - 23-25 Aug. 2001
- Indo-French symposium on HIV/AIDS and Tuberculosis - 7-9 Mar. 2002

Abbreviations

Acid Fast Bacilli	-	AFB
Annual Risk of Infection	-	ARI
Bacillus Calmette Guerin	-	BCG
Directly Observed Treatment Short Course	-	DOTS
District Tuberculosis Officer	-	DTO
Ethambutol	-	E
Functional Mutant Homozygous	-	FMH
High Performance Liquid Chromatography	-	HPLC
Interleukin –1 Receptor Antagonist	-	IL-1RA
Isoniazid	-	H
Isopropyl b-thiogalactopyranoside	-	IPTG
Lowenstein Jensen	-	LJ
Luciferase Reporter Phage	-	LRP
Mannose Binding Lectin	-	MBL
Multi Drug Resistant Tuberculosis	-	MDR TB
Natural Resistance Associated Macrophage Protein	-	NRAMP
Ofloxacin	-	O
Peripheral Health Institution	-	PHI
Pyrazinamide	-	Z
Restriction Length Fragment Polymorphism	-	RFLP
Revised National Tuberculosis Control Programme	-	RNTCP
Rifampicin	-	R
Streptomycin	-	S
Tuberculosis	-	TB
Tumor Necrosis Factor	-	TNF
Vitamin D Receptor	-	VDR

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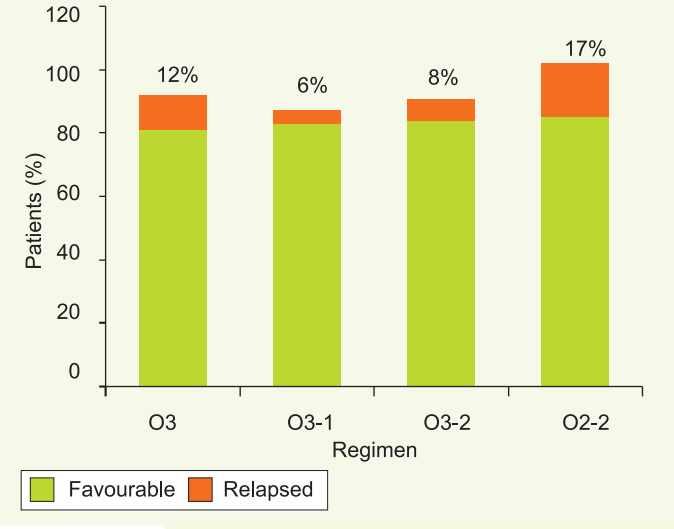
Chemotherapy

Controlled clinical trial for the treatment of sputum positive pulmonary tuberculosis with regimens containing ofloxacin

The follow-up of patients enrolled in the randomised clinical trial for the treatment of sputum positive pulmonary TB with regimens containing O, is continuing. Newly diagnosed patients with sputum positive pulmonary TB fulfilling the eligibility criteria were randomly allocated to one of the following four regimens: a) O, H, R and Z daily for three months (O3); b) regimen a, followed by H and R twice a week for one month (O3-1); c) regimen a, followed by H and R twice a week for two months (O3-2); or d) O, H, R and Z daily for two months, followed by H and R twice a week for two months (O2-2). A total of 529 patients were enrolled in this study. The study design, methodology, patient profile, bacteriological sputum conversion at two months, the results at the end of treatment and relapses up to 24 months after completing treatment were presented in the previous annual report. The regimen-wise relapse rates among patients with drug susceptible and H-resistant TB followed up for 36–60 months are shown in Figure 1.

Fig. 1 Relapse after treatment



Results at the end of treatment and follow-up up to 24 months after treatment have been published (Indian Journal of Tuberculosis 2002; 49: 27–38). Follow-up will continue for 60 months.

This study has shown for the first time that it is feasible to shorten the duration of treatment for smear positive pulmonary TB to less than six months. The four- and five-month regimens, with O for the first three months, were highly effective in the treatment of such patients.

Role of bronchodilators in improving the efficiency of diagnosis in smear negative pulmonary tuberculosis patients

The present RNTCP recommends three sputum smear examinations for diagnosis and management of TB based on smear results. For good sputum microscopy, prescribed methods of collecting sputum specimens, staining procedures and reading of sputum smears have to be accurately followed. The chance of missing diagnosis is high if any one of the instructions are not attended to. A patient may not be able to expectorate well if there is associated airway obstruction. In pulmonary TB, bronchospasm presenting as bronchial obstruction has been documented. The preceding supplement of bronchodilator therapy might facilitate effective expectoration and improve the sensitivity of smear microscopy. Hence, the role of bronchodilators in improving diagnosis in smear negative chest symptomatics was undertaken as a double blind study among outpatients attending the Tondiarpet Corporation Clinic, Chennai, where RNTCP has been implemented. A subsidiary objective was to assess the diagnostic yield of repeat sputum smear examinations after a course of antibiotics for persons with persistent symptoms.

Chest symptomatics of more than three weeks duration were subjected to three sputum examinations. If all were found to be negative, they were prescribed a course of antibiotics for 10 days and advised to report if the symptoms persisted. These patients when reported were randomly allocated to either the salbutamol or the placebo group. Salbutamol was administered in the dose of 2 mg twice daily for three days along with trimethoprim and co-trimoxazol under supervision. On the fourth day, repeat sputum examinations were performed on all the three specimens.

A total of 230 chest symptomatics were admitted to the study of which 24 were excluded for various reasons. The remaining 206 comprised a mixture of patients with TB and non-tuberculous respiratory infections. Among them, 40 (20 per cent) yielded positive cultures for *M.tuberculosis*. The repeat sputum examination after a course of antibiotics

plus salbutamol / placebo was positive for AFB in 26 (65 per cent) of the 40 TB patients.

Bronchodilator therapy has not been beneficial in improving the diagnosis. This study has shown that repeat sputum smear examinations have established the diagnosis in 65 per cent of TB patients.

Evaluation of an oral eight-month regimen with a non-rifampicin continuation phase in the treatment of sputum positive pulmonary tuberculosis

The currently recommended regimen under RNTCP in India is of six months' duration, thrice-weekly throughout, with an intensive phase of 24 doses of four drugs R, H, Z and E, followed by two drugs R and H for four months: $2RHZE_3/4RH_3$. Under programme conditions, the first phase is to be given under direct observation of a DOTS health provider, either at the treatment centre or in the home/ workplace of the patient (DOT). During the continuation phase, however, the patient receives only one dose under direct observation, while the other two doses for the week are supplied for self-administration, with proper instructions. This procedure could result in "concealed irregularity", with patients forgetting to take some of the doses meant for self-administration. It was, therefore, decided to evaluate a regimen with the same intensive phase, but with a non-R continuation phase daily for six months, with H and E given on a once weekly basis: $2RHZE_3$ / 6HE.

This regimen has the advantages that (a) even if a patient fails to take a few of the supplied doses, the proportion of treatment missed will be less in case of a daily phase than in an unsupervised thrice-weekly rhythm; (b) with a non-R continuation phase, there is less likelihood of emergence of R resistant strains of *M.tuberculosis*, even if a patient is irregular in drug self–administration; and (c) the continuation phase of HE can be supplied on a twice-monthly or even a once-monthly basis, especially in remote and inaccessible regions, where DOT may not be feasible for more than two months.

The primary aim was to assess its efficacy in terms of response at the end of chemotherapy and relapse rates over a two-year period. Patients aged 12 years or more with at least two sputum smears positive for AFB, and those who had not received more than one month of previous specific chemotherapy were admitted to the study.

In the second phase of this study, it was decided to admit another 200 patients, with the aim of assessing the impact on treatment outcome, of extending the initial intensive phase of treatment to patients with a positive sputum smear at two months. In these patients, the intensive phase of $RHZE_3$ was extended by 12 doses, as is being done under the RNTCP.

All patients have completed treatment and are being followed up in order to study relapse rates.

Randomised clinical trial to study the efficacy of intermittent regimens containing ofloxacin in the treatment of smear positive pulmonary tuberculosis

Encouraged by the results of the clinical trial of daily O regimens in the treatment of patients with sputum positive pulmonary TB, the centre is now carrying out a randomised clinical trial to study the efficacy of intermittent O containing regimens in the same category of patients. This study, being carried out in Chennai and Madurai, commenced in May 2001. Newly diagnosed patients with sputum positive pulmonary TB are randomly allocated to one of the regimens as shown in Figure 2.

a) 2 $OHRZ_3$ / 2 OHR_3 - O, H and R thrice a week for four months plus Z for the first two months

b) 2 $OHRE_3$ / 2 OHR_3 - O, H and R thrice a week for four months plus E for the first two months

c) 2 $EHRZ_3$ / 4 HR_3 - H and R thrice a week for six months plus E and Z for the first two months

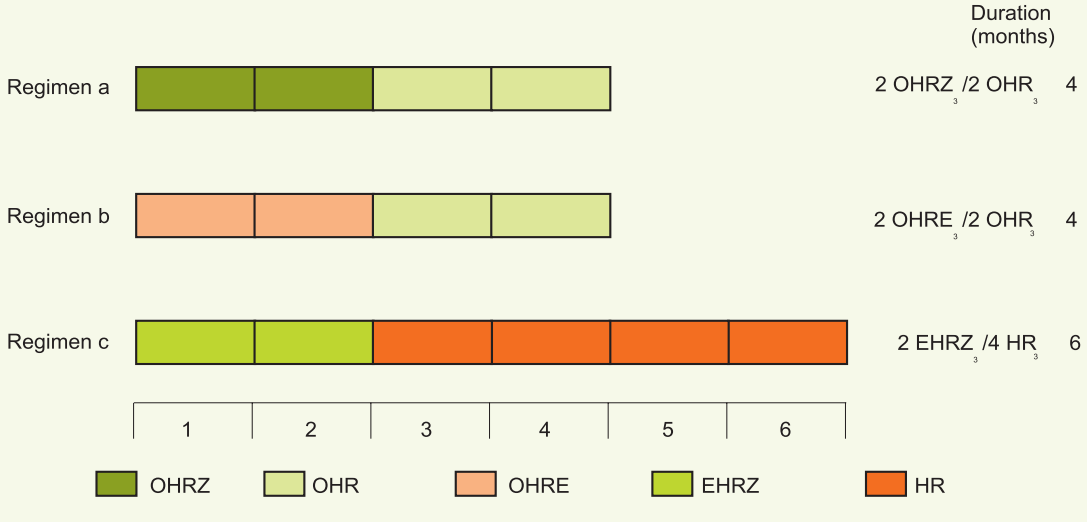
The outcome measures that will be estimated are bacteriological sputum conversion after two months of treatment, bacteriological status at the end of treatment, relapse during follow-up for 24 months and adverse reactions attributable to the treatment regimens. These outcomes will be compared between regimens and statistically analysed.

A sample size of 300 patients in each regimen is being aimed at. Initially, patients are being allocated to regimens a and c in the ratio of 2:1. After 225 patients are enrolled, patients will be allocated to all three regimens in the ratio of 2:4:3. A total of 112 patients have been allocated till now.

A study of the category I regimen (RNTCP) for the treatment of pulmonary tuberculosis associated with non-insulin dependent diabetes mellitus

Under RNTCP, category I regimen (2$EHRZ_3$/ 4RH_3) is recommended for new smear positive patients with diabetes mellitus. Hence, it was proposed to assess the efficacy, cure and relapse rates of the category I regimen among non-

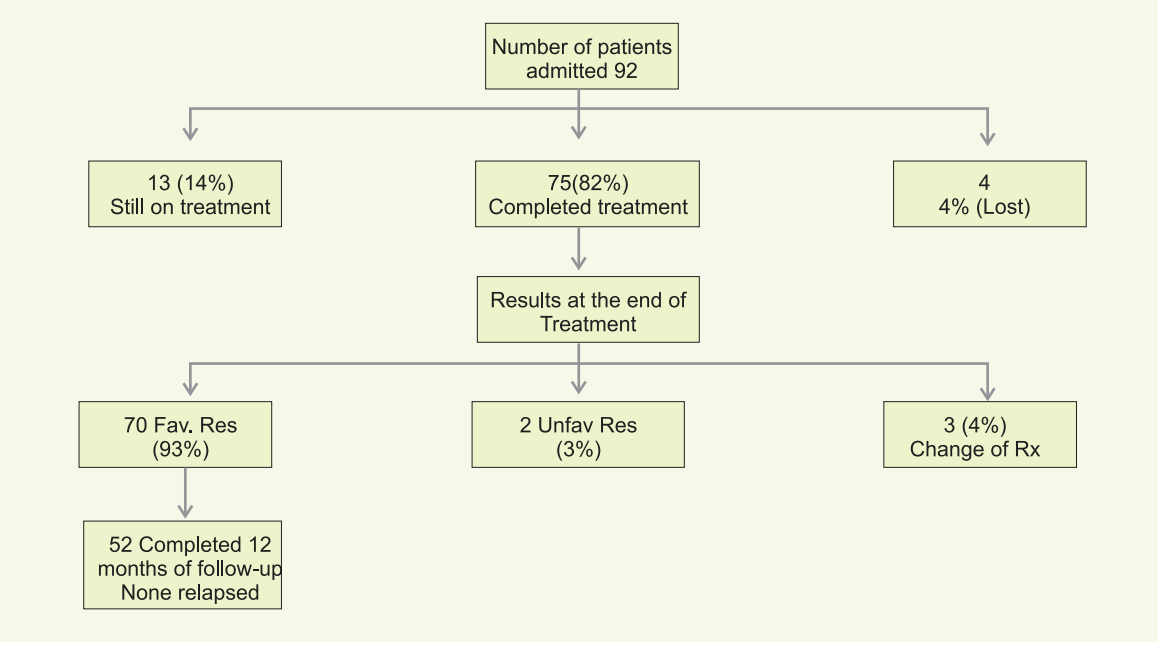
Fig. 2 Intermittent regimens containing ofloxacin



insulin dependant diabetes mellitus (NIDDM) for a period of three years from admission to study.

NIDDM patients attending the Government General Hospital, Kilpauk Medical College, Institute of Thoracic Medicine and the TRC suspected to have pulmonary TB were investigated at TRC. It is proposed to admit 100 patients to the study. All patients will receive the following regimen: ($2EHRZ_3/4RH_3$) - E 1200 mg plus H 600 mg plus pyridoxine 10 mg plus R 450 mg for patients weighing less than 60 kg and 600 mg for patients weighing 60 kg or more plus Z 1.5 g for all patients three times a week under supervision for two months followed by R plus H in the same dose for the next four months.

So far a total of 92 patients have been admitted of whom 79 per cent were males. Pretreatment mean glycosylated HB A1c was 9.7 per cent (range 5.9 per cent-14 per cent). The pretreatment drug susceptibility pattern is as follows; 94 per cent sensitive to all drugs, 5 per cent resistant to H and 1 per cent to R. Of 75 patients included in the analysis, 74 (99 per cent) became culture negative but 16 per cent remained smear positive by the third month of treatment and 70 (93 per cent) became culture negative at the end of the treatment. Results are shown in the following flow chart.



The study is in progress.

Staff list

M.S. Jawahar, M.D., M.Sc., D.L.S.H.T.M.
K. Rajaram, B.Sc., M.B.B.S., D.T.R.D.
Rema Mathew, M.B.B.S., D.C.H.
Paulin Joseph, M.B.B.S., D.D.
R. Balambal, M.D.
Usha Ramanathan, M.B.B.S., D.P.M.
D. Baskaran, M.B.B.S. D.T.C.D.
Sudha Ganapathy, M.A.
K.N. Gopilingam, C.R.A.
Jayalakshmi Vadivel, B.Sc.
B.V.S. Chalapathi Rao, B.Sc.
Arumaikannu Anbalagan, B.Sc.

A. Gunasundari, B.Sc.
G. Mangalambal, B.Sc.
C. Kavitha, B.Sc.
Vasanthira Patturaj
Susan Stella Bai
K.K. Valsamma
Emily Verghese
V. Meenakshi
P. Muthulakshmi
Manonmani Nathan
S. Padma
Padma Prakash
S. Chellam
R. Mirunalini
Victoria Kamalam Jayaraj
K. Rosily
Santhamma Asokan
K. Padmavathy
K. Sampooranam
G. Hemavathy
J. Vasantha Malathy
Catherine Bosco
Anna Antoney
Valliammal Jayaraj
A. Gomathy
C.T. Rajasekara Sastry
C. Arputha Mary
Nagalakshmi Janardhana Reddy
Geetha Ramani Shanmugam, Ph.D.
Jemima Sheila Fredrick
M. Rajasakthivel, M.A.
E. Thiruvalluvan, M.A.
Chandra Suresh, M.A.
D. Kalaiselvi, M.A.
P. Murugesan, M.A.

Contact persons

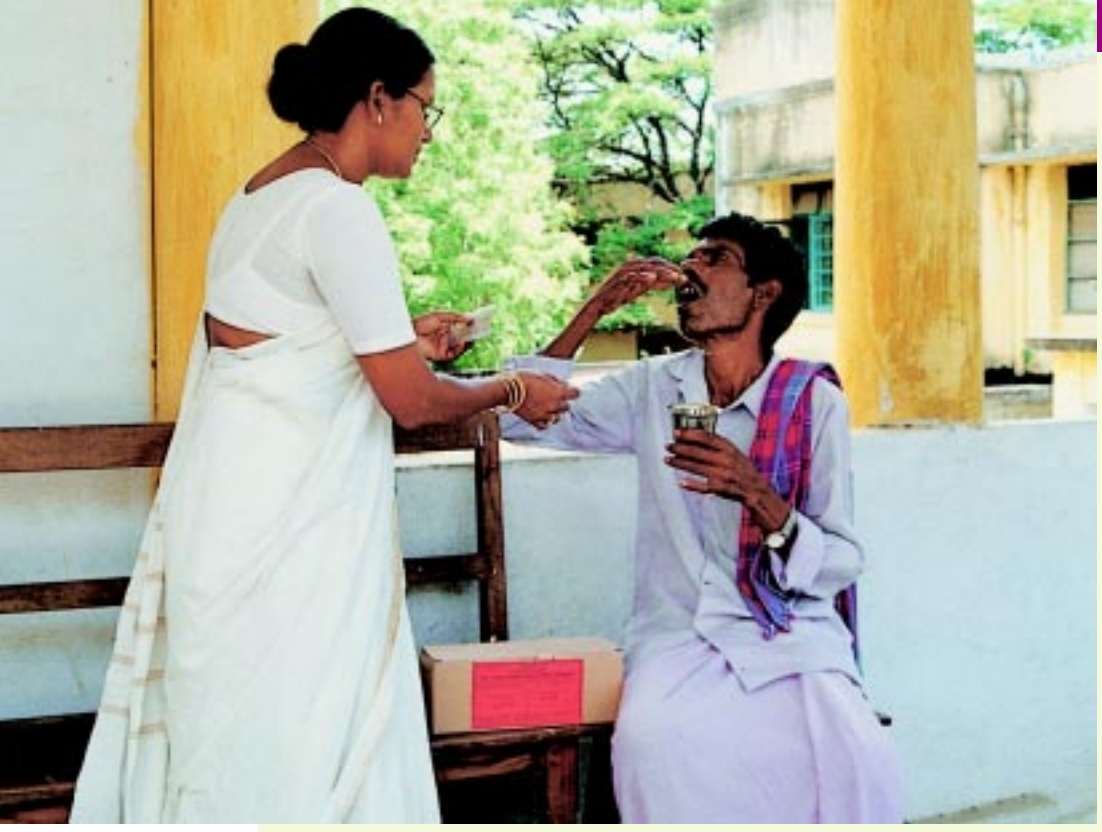
Design and conduct of randomised clinical trials
Gender and Tuberculosis
MDR-TB
Tuberculous lymphadenitis
Dr. M.S. Jawahar
trcicmr@md3.vsnl.net.in(clin1)

Biomedical ethics
Programme based randomised clinical trials
Dr. Rema Mathew
trcicmr@md3.vsnl.net.in(clin2)

Public private mix in TB control
Dr. R. Balambal
trcicmr@md3.vsnl.net.in(clin2)

Patient management in randomised clinical trials
Dr. K. Rajaram
Dr. Paulin Joseph
Dr. D. Baskaran
trcicmr@md3.vsnl.net.in(clin2)

Sociological aspects of tuberculosis
Mrs. Sudha Ganapathy
trcicmr@md3.vsnl.net.in(socialworker)



Operational Research

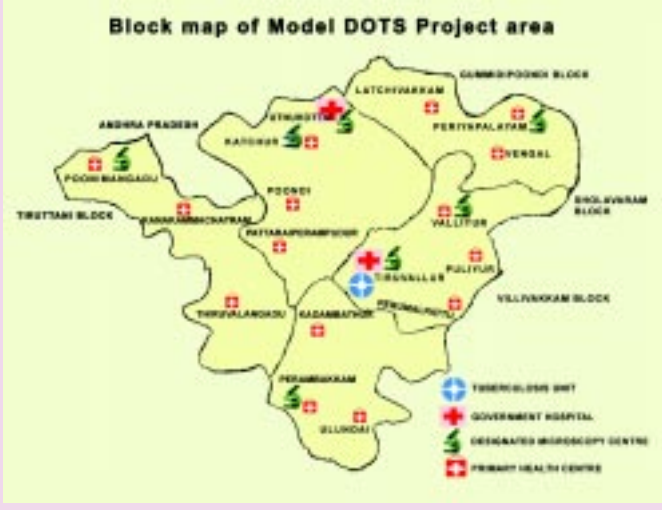
Establishment of a centre for DOTS demonstration, training and research in TB control

Directly Observed Therapy - Short course is universally accepted as the treatment strategy for TB control in both developing and developed countries. DOTS strategy makes it possible to carry out case finding, chemotherapy and patient monitoring effectively without hospital care. The Government of India is implementing this strategy in RNTCP in a phased manner since 1993. However, no information is available so far on the epidemiological impact of this strategy on the TB problem in a community. In order to understand this aspect, the Tuberculosis Research Centre has undertaken a project in collaboration with the Government of Tamil Nadu to establish a Model DOTS centre for DOTS implementation, TB control, training and research. It is proposed to measure the epidemiological impact of this strategy in the selected community over a period of at least 10 years. The project has been undertaken with technical support from WHO and financial support from USAID with the following objectives:

- Establish a centre for DOTS demonstration and training for DOTS implementation in a population of approximately 5 lakhs in Tiruvallur district in Tamil Nadu, India
- Strengthen the infrastructure at the state level, particularly in the districts surrounding the project area
- Assess the epidemiological impact of DOTS using ARI (tuberculin surveys), disease survey, RFLP and drug resistance surveys
- Conduct operational research on key aspects of the DOTS strategy

Project area

Five panchayat unions in Tiruvallur district covering a population of 5,88,000 were selected for the Model DOTS project. There are 218 villages in these blocks. This is the same area where the famous "Chingleput BCG Study" was conducted. The availability of information of a demographic pattern and epidemiology of TB for a long period prompted the choice of this site. Since the area has been under observation for a long period, the epidemiological impact of DOTS programme can be effectively measured here.



DOTS implementation

After completing the necessary civil works for microscopy centres and training of medical and paramedical staff in the area, service delivery was initiated on May 3, 1999. Up to December 31, 2001, 2,416 patients had been admitted for treatment. Details of these patients are given in Figure 3.

Annualised case detection rate

The quarterly wise annualised case detection rate based on the new sputum smear positive cases started on treatment in MDP area is shown in Figure 4.

Fig. 3 Categorywise distribution of patients

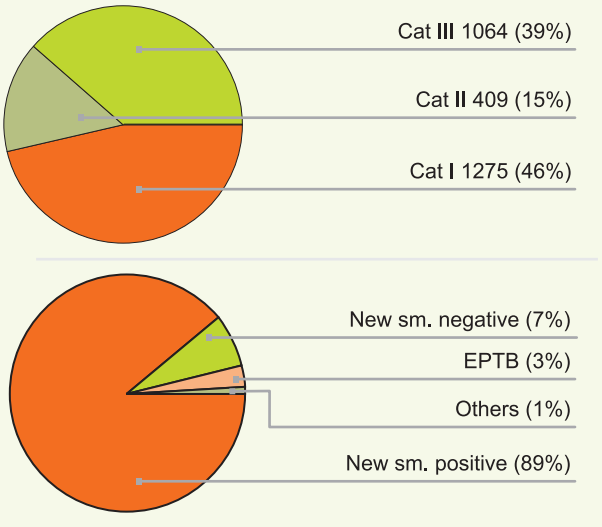
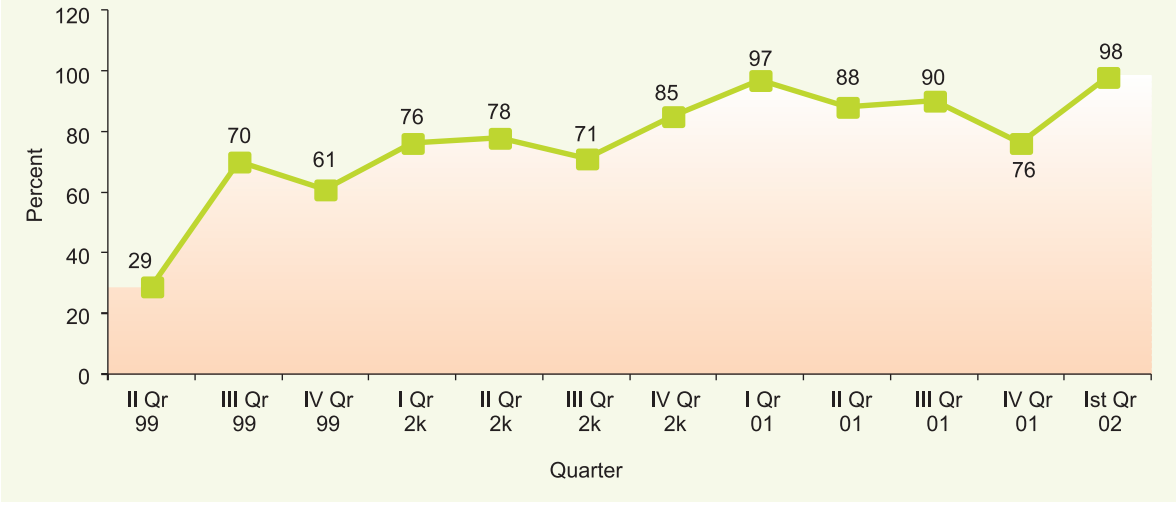


Fig. 4 Annualised case detection rate



Sputum conversion and cure

The sputum conversion and cure rates for new smear positive cases are shown in Figure 5.

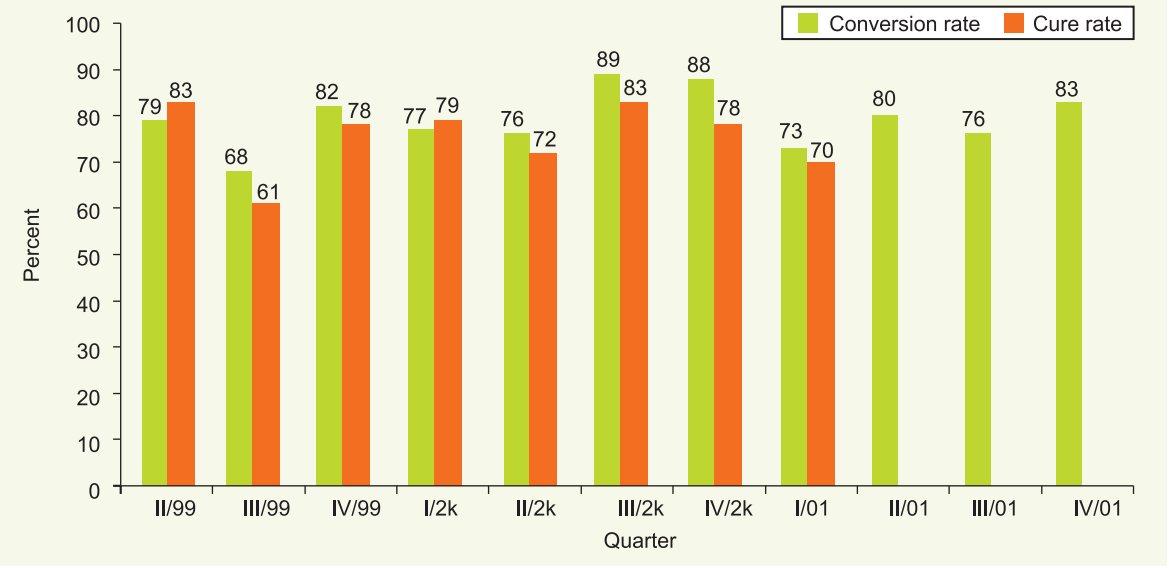
DOTS demonstration and training

Medical and paramedical trainees are taken regularly to the area for field-level training in RNTCP. So far, 324 medical officers (MO trainers), including 13 WHO-RNTCP consultants, 100 medical officers, 228 senior treatment supervisors, 197 senior tuberculosis laboratory supervisors and 28 laboratory assistants, have been given field training in the area.

State strengthening

The centre is providing all assistance required by state-level officers for TB control activities. We are involved in training of the trainers from various districts in Tamil Nadu. The centre participated in the workshop to involve medical colleges of Tamil Nadu in RNTCP. A review meeting was conducted with the District Tuberculosis Officers (DTO) of various implemented districts of Tamil Nadu.

Fig. 5 Quarterly conversion and cure rate



The centre has been responsible for strengthening supervision by the DTO of Tiruvallur. Through periodic meetings with the STS and STLS, we have streamlined the recording and reporting system in the district. The centre has helped in advocacy at various levels, medical colleges, Employees State Insurance Hospital and Southern Railway Hospital. The last two have started implementing RNTCP.

We have printed brochures for DOT providers, patients, their families and NGOs describing their role in the control of TB. We have also come out with a poster on the disease for display at the community and peripheral health institution (PHI) levels.

Preparation of additional training aids

The centre has prepared visual presentations on all the 10 training modules to familiarise the participants with the modules. We are in the process of preparing a re-training module and assisting in the preparation of a condensed module of 5–10 that will help the PHI medical officers involved in the programme to have a clear idea as to how programme performance is evaluated.

Epidemiological impact study

In order to assess the epidemiological 25 impact of DOTS implementation, the first resurvey to estimate the prevalence of TB disease and infection in the project area has been started.

Progress of the disease survey

The aim is to estimate the prevalence and incidence of disease, and to measure the epidemiological impact of the DOTS strategy over a 10-year period. The baseline survey has been completed and the first resurvey has been initiated. A random sample of panchayats/ urban units was included for the first resurvey. The survey was started in June 2001 and has been completed in the Kadambathur panchayat union. The survey is now in progress in the Tiruvalangadu panchayat union.

Baseline survey

The baseline survey was completed in June 2001. The coverage for the survey is given in Table 1.

The first resurvey was started in June 2001 and so far 14 panchayats have completed X-ray. Coverage was around 90 per cent for all investigations, namely symptoms, X-ray and sputum examination as seen in Table 2. The 27 persons diagnosed were referred for treatment.

Table 1 Coverage for baseline survey

Sex	Eligible for screening	Examined (%)	Eligible for sputum	Examined (%)	No. of cases/1000
Male	47762	41981 (88%)	6290	6015 (96%)	444 (10.6)
Female	49823	46851(94%)	4036	3936 (98%)	98 (2.1)
Total	97585	88832 (91%)	10326	9951 (96%)	542 (6.1)

Table 2 Coverage for disease survey

Activities	Population
Eligible for symptom and X-ray	19067
Symptom screening	17718 (93%)
X-rayed	17566 (92%)
Sputum eligible	2725
Sputum collected	2595 (95%)
Tuberculin tested and read	7996*

* Tuberculin testing was repeated only for those who were negative in the baseline survey.

Tuberculin survey

Progress of the survey

The aim of the survey is to provide a series of ARI estimates over a period of time that will help measure the epidemiological impact of implementation of the DOTS strategy. The second survey on ARI has been started in a mutually exclusive random sample of panchayats and urban units to cover an estimated population of 45,000 as done in the baseline survey. Results of the baseline survey are summarised in Table 3.

Table 3 Baseline survey of ARI

Age	No. Examined	Infected No.	Infected %	ARI
2m – 4yrs	5386	179	3.2	1.3
5 – 9 yrs	7473	739	9.9	1.4
10 – 14yrs	9456	1911	20.2	1.8
2m – 14yrs	22312	2820	12.9	1.8

So far in the first resurvey, 13 panchayats have been covered for the tuberculin survey. Of the 8,344 children tested, reaction was read in 7,908 (94.8 per cent) children.

Operational research

Washing of new microscopic glass slides in dichromate solution does not influence sputum AFB smear results

Acid-fast bacilli microscopy is an essential component of DOTS. A large number of sputum smears are being made in DOTS programmes the world over. Fungal and bacterial spores or dust particles on microscopic glass slides can lead to false positive results for AFB. Greasy material on glass slides can make preparation of smears difficult by preventing firm fixation of sputum smears on the slides. Therefore, in many institutions, new microscopic slides are washed in dichromate solution before they are used in sputum microscopy. However, WHO guidelines do not include this procedure. Therefore, a comparison of smear positivity for AFB following these two procedures was undertaken to determine the influence of washing of slides in dichromate solution on smear results, if any.

Two direct smears were prepared from each of 1,750 sputum samples. One was made on the dichromate solution-cleaned new glass slides and the other was made on unwashed new glass slides. The smears were blinded and examined.

The results reveal that washing of new glass slides in dichromate solution is not essential for AFB microscopy.

Evaluation of "two reagent cold staining method" for acid-fast bacilli

The Ziehl-Neelsen staining procedure involves a number of laborious steps such as filtering carbol fuchsin before every use, applying external heat while staining with carbol fuchsin, decolorisation with acid and counter staining with methylene blue which could be cumbersome from the laboratory technician's point of view. Hence, several modifications of the ZN method were explored in the past to simplify the procedure with varied success. A commercially available "two-step cold staining method" for AFB in sputum smears was evaluated against culture as gold standard.

Two smears were prepared from each of 244 sputum samples; one was allotted to the cold method ("two reagent cold staining method") and the other to the ZN method. The smears were blinded and read. All the samples were processed by modified Petroff's method for culture of *M.tuberculosis*.

The concordance (smear grade one above and one below) between the methods was 90 per cent (kappa = 0.7). The performance of cold method and ZN method was similar when their smear results were compared with culture results (cold method vs culture, kappa = 0.61; ZN method vs culture, kappa = 0.67).

The results of the study suggest that the "two reagent cold staining method " is as sensitive and specific as ZN method. However, large-scale multicentric studies in different climatic conditions need to be conducted to assess its efficacy in the diagnosis of pulmonary TB.

The inefficiency of 0.3 per cent carbol fuchsin in Ziehl-Neelsen staining for detecting acid-fast bacilli

In the standard ZN method, the concentration of carbol fuchsin is one per cent. But, WHO guidelines recommended concentration of 0.3 per cent. Since the efficiency of 0.3 per cent carbol fuchsin over one per cent carbol fuchsin is not known, a systematic study was carried out.

Two smears were prepared from each of the 586 sputum samples; one was allotted to standard ZN method in which 1 per cent carbol fuchsin was used and the other to the modified ZN method in which 0.3 per cent carbol fuchsin was used. The smears were blinded and read. All the samples were processed by Petroff's method for culture of *M.tuberculosis*. The sensitivity of modified ZN and standard ZN methods were 72 per cent and 84 per cent, respectively. The modified ZN method was less efficient than the standard ZN method; it failed to detect 20 per cent of the cases found positive by the latter and 11 per cent of the culture proven cases.

The results of the study suggests that WHO recommendation of 0.3 per cent carbol fuchsin in ZN method for staining AFB needs reconsideration.

Evaluation of phenol ammonium sulphate sedimentation smear microscopy method for the diagnosis of pulmonary tuberculosis

The sensitivity of ZN staining method varies and depends upon proper preparation of the smears, good staining technique, careful examination of smears and the availability of a good microscope. Improved smear microscopy methods feasible in field conditions are needed to improve the diagnosis of pulmonary TB. In developing countries, laboratory technicians sometimes tend to sidestep sputum examination owing to apprehensions about the infectiousness of samples and due to the cumbersome way of preparing direct smears from the mucus portion of the sample. A study was, therefore, undertaken to evaluate the sensitivity,

specificity and acceptability of the phenol ammonium sulphate (PhAS) sedimentation smear microscopy method.

A total of 2,400 sputum samples were studied. Each sputum sample was divided into two parts and randomly assigned; one to the modified Petroff's method for culture of *M. tuberculosis* and the other to the PhAS sediment smear method, from which direct and sediment smears were prepared. All the smears were stained by the ZN method and were coded for reading. In all, 547 specimens were culture positive. The sensitivity of PhAS and direct smear methods was, 85 per cent (465/547) and 83 per cent (454/547) respectively (p =0.7), and the specificity of each of the methods was 96 per cent.

PhAS method was found to be as sensitive and specific as the direct smear method and it was better accepted by laboratory technicians. Also, it is safer to use but necessitates overnight sedimentation, which delays reporting of results until one day after sputum collection.

Molecular epidemiology

The centre is undertaking RFLP studies on positive cultures obtained from patients undergoing treatment and those isolated in the survey in order to understand molecular epidemiology in the area and to monitor the same over time.

RFLP patterns

RFLP analysis by IS6110 probe identified 4 (1 per cent) strains with no copy, 154 (41 per cent) strains with a single copy, 99 (26 per cent) strains with 2-5 copies and 101 (32 per cent) strains with more than 6 copies. In all, 271 distinct patterns were identified among 378 subjects. The median number of bands was 2 (range 0-17). Analysis with IS6110 probe revealed that 225 (60 per cent) of the 378 patients matched DNA fingerprints of at least one other patient-isolate in the study. These 225 patients were in 17 clusters and of them, 143 were in a single cluster having a single band. Since the IS6110 probe has lower discriminative power in single copies, secondary fingerprinting was performed using DR probe.

Combined analysis of fingerprints by both IS6110 and DR probes identified 236 patients with a unique strain and 142 (38 per cent) patients in one of the 35 clusters. The number of patients in each cluster ranged from 2-25. Fifteen of the 35 clusters had two patients and 11 clusters had more than four patients.

Patients who had similar DNA fingerprints are being interviewed by home visits to identify any epidemiological links.

Analysis of clusters

One hundred and thirty-one of the 142 who were in one or more clusters could be contacted for epidemiologic investigation. Of them, 21 (16 per cent) reported acquaintance with one or more patients in the same cluster. Eleven of the 35 clusters had four or more patients and were analysed in detail. Of the 87 patients in 11 clusters, all but 4 patients in cluster 26 had strains with one band using IS6110 probe. The largest cluster (cluster 2) had 25 patients; the median age of these patients was 45 years and 56 per cent were men. Of the 25 patients in the largest cluster, 22 were interviewed; three pairs of patients (6) named one another as contacts while six patients had been hospitalised in one TB sanatorium in the past five years. In addition, although seven patients were alcoholics or reported high-risk sexual contact, they were not otherwise linked epidemiologically. In the second largest cluster (cluster 7, n=10), patients were slightly older (median age= 49 years) and four patients reported being hospitalised in one of the two TB hospitals. In cluster 3 and 6, each with six patients, risk of being clustered was associated with case detection by a house-to-house community survey ($p<0.05$).

Table 4 Distribution of patients by category and bacteriology results

Category	Total Patients	S+C+	S-C+	S+C-	S-C-	Total S+	Total C+
1	1027	615	218	32	162	647	833
2	311	195	48	7	61	202	243
3	797	27	94	16	660	43	121

Private pharmacies in tuberculosis control – a neglected link

This study highlighted the need and the potential of private pharmacies for participation in the TB control programme. In most settings in India, private pharmacies dispense prescriptions for anti-TB drugs given by private practitioners. In a cross-sectional study, we assessed the dispensing practices for TB and knowledge on the TB programme of 300 pharmacies. In all, 2,800 prescriptions were dispensed monthly by the pharmacies. Doctors' prescriptions were for months but half the patients bought drugs one dose at a time for self-administration. This practice can promote drug resistance.

Although 95 per cent of pharmacists were not aware of the TB programme, majority (97 per cent) were willing to learn and contribute towards control of the disease.

Drug resistance surveillance in MDP area

This study was undertaken to obtain information on the drug susceptibility pattern among patients admitted for treatment in the project area and to study the profile over a period of time to ascertain the impact of the programme.

Two additional sputum samples were collected from patients started on treatment in the Model DOTS area, preferably within a week after starting treatment.

The collection of sputum samples for culture and sensitivity was started in August 1999. So far, pre-treatment sputum specimens have been collected from 1,966 patients. Bacteriological results were available for 1856 patients. Of these, culture results are available for 1,017 patients and drug susceptibility pattern for 856 patients. Category-wise distribution of the culture results and susceptibility pattern are given in Tables 4 and 5.

Table 5 Drug susceptibility profile

	CAT I & III		CAT II	
	No	%	No	%
Total tested	866		214	
Fully sensitive	749	86.5	140	65.4
Any resistance	117	13.5	74	34.6
Res. to: S	28	3.2	4	1.9
H	45	5.2	29	13.6
R	3	0.3	–	–
SH	29	3.3	16	7.5
HR	5	0.6	17	7.9
SHR	7	0.8	8	3.7
Any H res.	86	9.9	74	34.6
Any R res.	15	1.7	25	11.7
Any HR res.	12	1.4	25	11.7

Of the 866 patients admitted to category I and III and for whom sensitivity results were available, 749 (86.5 per cent) were sensitive to all drugs, 28 resistant to S alone, 45 to H alone and only 12 (1.4 per cent) had resistance to H and R(MDR TB). Among 214 category II patients, 140 (65.4 per cent) were sensitive to all drugs, 29 had resistance to H and 25 (11.7 per cent) had MDR TB. Thus, of the 1,080 patients admitted for treatment and for whom sensitivity results were available, 37 (3.4 per cent) had MDR TB.

Involving private practitioners in the RNTCP

A feasibility study to involve private practitioners has been initiated in May 2001.

After listing the private laboratories in the area, we trained 10 laboratory technicians from them. There are 78 allopathic medical practitioners in that area and they were given an orientation to RNTCP on two occasions followed by focus group discussions to formulate the study design. A one-to-one meeting was held with the practitioners by the TRC medical officer and they were given a referral book of sputum examination forms with serial numbers. They were also given the option of referring the TB suspects either to one of the private laboratories or to the government hospital at Tiruvallur. For patients diagnosed, the treatment cards were made in duplicate at the Tiruvallur Government hospital and the first dose of treatment given at the TB unit (TU). Further management of the patient was decided in consultation with the private practitioner. The treatment box was handed over to the DOTS provider and the provider trained on administration of treatment and the card maintenance.

A total of 43 private practitioners have been enrolled. Also, 238 symptomatics have been referred, 65 have been diagnosed to have TB of which 34 were smear positive, 26 smear negative and 5 extra-pulmonary and all have been started on treatment.

Economic evaluation of Revised National Tuberculosis Control Programme

The main objective of this study was to assess the costs (economic and non-economic) involved in patients taking treatment in a DOTS area and compare the same with that from a non-DOTS area. This study is being carried out in the Model DOTS project area where RNTCP is being implemented (Tiruvallur district) and also in an adjacent area in the same district where NTP is implemented (control area). Patients diagnosed as having TB and started on treatment during the second half of the year 2000 were interviewed using a structured pre-coded interview schedule. Health status was measured for physical functioning, social functioning, role limitations due to physical and emotional problems, mental health, energy and vitality, pain and general health perception.

A total of 496 patients in the DOTS area and 441 in the non-DOTS area of Tiruvallur district, registered during June-December 2000, were interviewed to assess (i) direct medical costs, i.e., consultation fees for doctors, money spent for investigations and drugs; (ii) direct non-medical costs of travel, hospitalisation and special food; (iii) indirect costs due to loss of wages. These costs were calculated for the pre-treatment period–from symptoms to diagnosis–as well as during the treatment period. The study is in progress.

Involvement of self help groups in the TB Control Programme

A feasibility study on involvement of Self Help Group (Magalir Thittam) in the TB Control Programme has been initiated. SHGs are a willing force available in all villages to give voluntary services for health programmes. It is proposed to explore the possibility of involving self-help group volunteers, in sensitising the community on TB and utilising them as DOT providers for TB patients on treatment. Sensitisation, identification of DOT volunteers and establishing links with the multipurpose volunteers in the Model DOTS area and sensitisation to SHG groups in other areas in Tiruvallur district have been completed. So far, 1,234 women have been sensitised and 130 women have been trained as DOTS providers.

Delay and drop out: barriers to tuberculosis diagnosis in disease endemic countries

Due to the interplay of patient, technical, operational and health system factors, case detection remains a major obstacle to TB disease control. A better understanding of these factors and the frequency and timing of diagnosis delay and default is essential before implementing

interventions, such as new diagnostic tools. This project will quantify and identify factors associated with diagnostic delay and default in four geographically diverse disease endemic countries. The significance of delay will also be measured in terms of economic loss, morbidity and treatment outcome of newly-diagnosed TB patients. India has been chosen as one of the centres for carrying out this study for which the protocol and forms have been finalised.

Course of action taken by the smear negative chest symptomatics

In RNTCP, the diagnostic algorithms for smear negative chest symptomatics requires prescribing antibiotics for 2-3 weeks followed by chest X-rays if symptoms persist. The patient is started on treatment if the X-ray is suggestive of active TB.

Currently there is no mechanism to track whether the smear negative patients report back to the health system for further evaluation and whether the diagnostic algorithm is followed strictly.

We are undertaking a prospective study on 600 smear negative symptomatics to find out the course of action taken by them and to assess whether the diagnostic algorithm is followed. When a patient is reported as negative by three smears and comes to collect the results, a fresh specimen will be collected for culture and sensitivity. A month later, they will be visited at home to find out the action taken by the patient during the intervening period.

So far, sputum specimens have been collected from 154 smear negative symptomatics who had come to collect their results and 76 have been visited at home. The study is in progress.

A multicentric study to estimate the prevalence of chest symptomatics attending various health facilities

An accurate estimate of the prevalence of chest symptoms among patients attending health

facilities is needed to evaluate if case detection efforts are adequate. The objectives of this study are to obtain information on the prevalence of chest symptoms among adult out-patients and to compare the efficiency of TB case detection among patients with coughs of different duration (2 vs 3 weeks).

The study will be conducted in six selected districts in the states of Rajasthan (Jaipur, Jodhpur and Udaipur), Tamil Nadu (Kancheepuram and Tiruvallur) and Maharashtra (Pune). In each of the selected districts, four primary-level and three secondary-level health facilities will be selected. A sample size of approximately 28,800 patients (14,400 each for primary and secondary-level facilities) is required to obtain about 30 TB cases each at the two facilities, assuming a yield of two smear-positive TB cases per 1000 new adult out-patients screened.

The expected outcome indicators are prevalence of chest symptoms among adult patients and smear positive cases by age, sex, duration of cough and type of facility.

The study is to be conducted in two alternating quarters, so as to assess seasonal variation if any. The first phase of the study has been completed and the second phase is in progress.

Understanding gender inequalities in TB epidemiology and control

We are analysing the data on prevalence of infection and disease from community surveys and among TB patients diagnosed and treated at government health facilities in the project area to understand the gender differentials in various parameters.

Staff list

T. Santha Devi, M.B.B.S., D.T.C.D.
A. Thomas, M.D., Dip. in Lep.
Rajeswari Ramachandran, M.D., D.M.(Neuro.), Ph.D.
Rani Balasubramanian, M.D., D.G.O.
K.C. Umapathy, M.B.B.S.
P. Paul Kumaran, M.B.B.S.
Niruparani Charles, B.A., D.S.S.A.
K.J. Jaganatha Rao, M.A.
S. Radhakrishnan, B.A. (STS).
E. Prabhakaran, D.M.L.T (STLS)
V.S. Sukumaran, M.A.
M. Kalyanaraghavan, M.Sc.
M. Muniyandi, M.A., M.P.S., M.Phil.

Contact persons

Operational Research and Training

Dr. T. Santha Devi
trcicmr@md3.vsnl.net.in(mdp1ts)

Dr. Aleyamma Thomas
trcicmr@md3.vsnl.net.in(mdp1ts)

Dr. Rajeswari Ramachandran
trcicmr@md3.vsnl.net.in(rntcptrg)

Dr. Rani Balasubramanian
trcicmr@md3.vsnl.net.in(rntcptrg)

Dr. K.C. Umapathy
trcicmr@md3.vsnl.net.in(rntcptrg)

Epidemiological Research Management

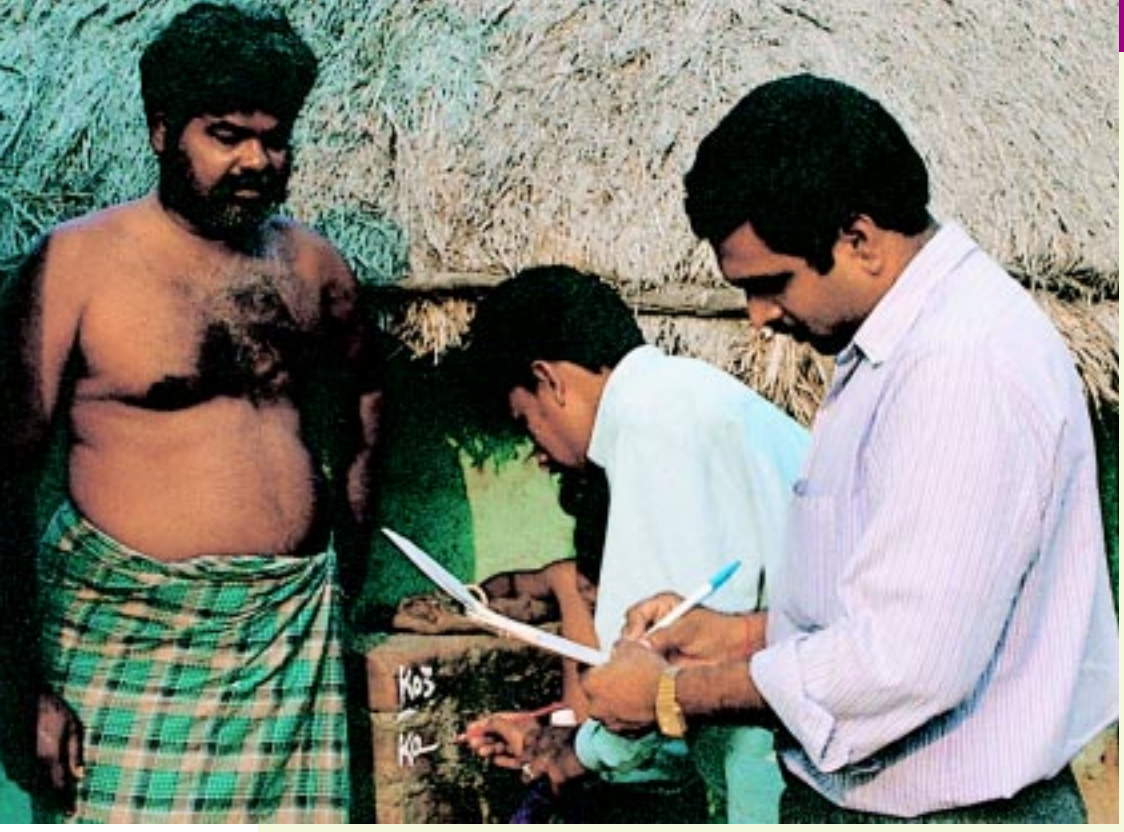
Dr. P. Paul Kumaran
trcicmr@md3.vsnl.net.in(mdp2pk)

Bacteriological Studies in DOTS Project

Dr. N. Selvakumar
trcicmr@md3.vsnl.net.in(bact2)

Statistics

Dr. V. Chandrasekaran
trcicmr@md3.vsnl.net.in(mdp2pk)



Epidemiology

Epidemiological impact study

In order to assess the epidemiological impact of DOTS implementation, it was proposed to conduct a series of disease and tuberculin surveys to estimate the prevalence/ incidence of TB disease and infection in the DOTS implemented area.

Disease survey

A baseline survey was carried out in a random sample of population of about one lakh covering all the five panchayat unions of the project area. Two screening methods were employed for case detection among persons aged 15 years and above. This population was tuberculin tested with 1TU RT23 in order to assess the infection among the adult population. Two sputum specimens collected from those who were either symptomatic or had abnormal chest X-rays were processed for smear by fluorescent microscopy, culture and susceptibility testing.

When one or both specimens tested positive, either by smear or culture, the individual was referred for a third specimen in order to refer for treatment according to RNTCP procedures under Model DOTS. The patients referred for treatment based on three specimens were motivated to visit the nearest PHC.

Coverage was around 90 per cent for all investigations namely symptoms, X-ray and sputum examination as seen in Tables 6 and 7.

Table 6 Coverage of disease survey

Activities	Population
Census covered	136947
Eligible for symptom/X-ray	97585
Examined for symptom/X-ray	88862
Tuberculin tested/read	80713
Sputum eligible	10450
Sputum collected	10040

Table 7 Bacteriological results

Screening method	S+C+	S-C+	S+C-	S-C-	Total
Symptom only	28	57	17	6043	6145
X-ray only	96	142	7	2319	2564
Symptom & X-ray	120	74	12	1037	1243
Total	244	273	36	9399	9952

The prevalence of bacillary tuberculosis was estimated to be 6.5 per 1000 population.

Tuberculin survey

A tuberculin survey was carried out in a representative sample of children aged 2m – 14 years. All eligible children were tuberculin tested and the reaction size was read after 48 to 96 hours. Presence of BCG scar was also ascertained. Prior consent was obtained before subjecting the children to testing from their parents. In all, 40,147 children were tuberculin tested and read for reaction. Among the children for whom BCG scar status was available, 17,837 (44.4 per cent) had a BCG scar. Table 8 shows the infection rate and ARI among children in different age groups without BCG scar.

It can be observed that the prevalence of infection as also of ARI increased as the children grew old. The overall prevalence of infection was 14.2 per cent among children aged 2m -14 years, ARI being 2.0 per cent.

Table 8 Prevalence of infection and annual risk of infection among children without BCG scar

Age	No. Examined	Infected		ARI
		No.	%	
2m-04years	5381	196	3.6	1.5
05-09years	7473	812	10.9	1.5
10-14years	9456	2166	22.9	2.1
Total	22310	3174	14.2	2.0

Table 9 Overall BCG coverage in South Zone

District Name	No. Tested	No. Read	Scar	
			Present	Absent
Dakshina Kannada	8012	7746	5651	2078
Medak	6386	6143	3242	2881
Belgaum	10654	10223	6274	3945
Kanyakumari	4554	4440	3549	878
Malappuram	8975	8745	5192	3543
Chingleput	14043	13549	8836	4604
Total	52624	50846	32744	17929

Note: For 173 children, BCG scar status was doubtful.

Follow-up survey

Resurvey was started in the same population in June 2001 after the baseline survey. Among 20,782 persons aged 15 years and above eligible for examination, 19,434 were covered for X-ray/symptom and 96 cases were detected. During this survey among the adult population, those not infected in the previous survey were attempted for tuberculin testing. Thus, 8,832 people were tuberculin tested and read.

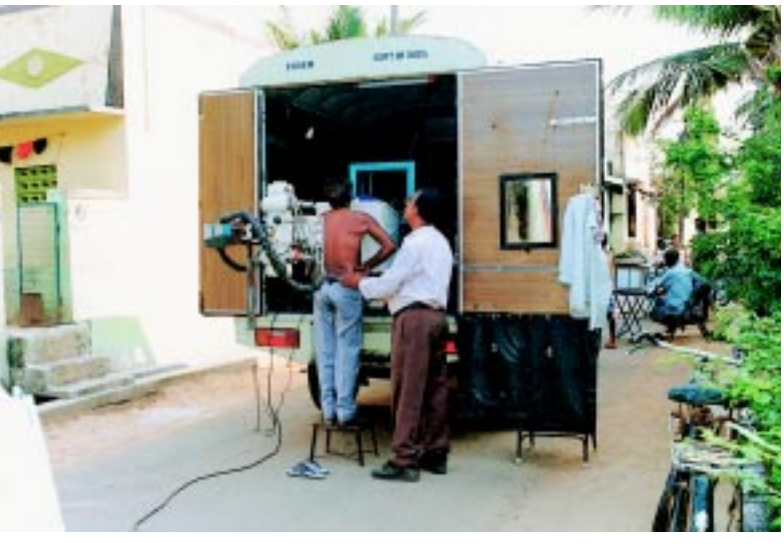
Tuberculin survey was also started in a mutually exclusive random sample of children aged 0-14 years old. So far, 7,703 children have been covered for testing and reading.

National Sample Survey

A nationwide tuberculin survey was started to assess the epidemiological situation of TB in different zones of the country by estimating the prevalence of infection and thereby computing the ARI. The country was divided into four zones namely; east, west, north and south. The survey in the south zone (Karnataka, Andhra Pradesh, Kerala and Tamil Nadu) was undertaken by the centre in 600 clusters in selected districts.

The sample size for each zone was estimated at 51,000 in order to obtain 12,000 test-read children without a BCG scar.

The survey was started in the south zone in January 2000 and completed in May 2001. A total of 52,951 children were registered and from among the 52,624 children tested, the reaction was read in 50,846. The BCG coverage varied from 52.9 per cent in Medak district to 80.2 per cent in Kanyakumari. The overall BCG coverage was 64.8 per cent (Table 9).



The prevalence of infection by weighted analysis was 6.5 per cent and ARI was computed as 1.2 per cent for the south zone.

Assessment of X-ray readers

The centre has generated voluminous data over the last three decades from epidemiological surveys. In such surveys, X-ray examination is one of the screening methods employed to collect sputum for detection of bacillary forms of TB. Hence, there has always been a need to train and assess the clinicians in reading Mass

Miniature Radiography (MMR) films used in the survey.

Recently, such an exercise was carried out in assessing three clinicians of this centre. Initially, they were given intensive training by a standard reader with the help of the manual and other materials in categorising each photofluorograms as normal, non-TB, possible or probable TB. An interim evaluation during the training period showed that the extent of agreement for sputum eligibility between the readers and the standard reader after adjusting for chance agreement was 27–30 per cent. Over-reading was to the extent of 50–70 per cent and under-reading 2–5 per cent. During the assessment, 45 rolls consisting of about 1800 films were given to all the readers. Although the exact agreement was low (30–40 per cent), the chance corrected agreement for sputum eligibility was about 60 per cent. The over- and under- readings were also substantially reduced. Efforts are on to assess the internal consistency.

Electronic data processing

Electronic data processing division is continuing data entry, verification and management support for various studies undertaken at the centre. The routine data output and regular backup of data is being done. The quantum of documents entered and verified from January 2001 to March 2002 are shown below:

No. of documents entered : 3,25,088
No. of documents verified: 3,23,016

Computerisation of treatment cards of the Model DOTS project has been completed. The pre-printed documents and required lists are being supplied to the field division to start the first resurvey examination (disease survey).

The LAN computer system in the division enables access of information through the ISDN internet facility and aids communication among staff through the intranet facility. A 64 kbps radio frequency (RF) connectivity for internet by NIC Services Incorporated (NICSI) under the sanction of ICMR has been installed.

Staff list

C. Kolappan, M.B.B.S., M.Sc.(Epid)
K. Sadacharam, M.B.B.S., D.P.H.
P.G. Gopi, M.Sc.
R. Selvaraj, M.Sc.
R. Subramani, M.Sc.
R.S. Nagabooshana Rao, B.Com.
K.R. Bhima Rao, B.Sc.
V. Venkatesh Prasad, B.Sc., B.G.L.
G. Baskaran, M.Sc.
S.I. Eusuff, B.Sc., B.L., PG Dip.PM & IR, M.A. (Sociology)
J. Devan, M.Sc.
T. Nataraj, M.Sc.
K.R. Ravichandran, B.Sc.
Malathy Parthasarathy, B.Sc.
G. Komaleeswaran, M.Sc.
R. Ponnuswamy, M.Sc.
S. Boopathy, B.Sc.

K. Gopala Jetty
G. Prabhakar, B.A.
E. Sakthivelu, M.A.
K. Balasubramaniam, B.Sc.
S. Gopalakrishnan
V. Subramanian
T. Raman
N. Ravi, D.E.E., P.G.Dip.Med.Eq.
R. Chandra Mohan, B.Sc.
M.S. Govindaraj, B.A.
S. Ranganathan, B.Sc.
K.V. Venkataramu, B.Sc.
Abdul Khudoos, B.Com.
A. Narasimhan
C.R. Sudeendra, B.Sc.
R. Sashidharan, B.Sc.

B.S. Phaniraj, B.A.
S. Sathiamurthy, B.A.
N. Ganesan, B.A.
L. Krishnamacharya, B.Sc.
S. Radhakrishnan, B.A.
Annamalai Baskaran, B.Sc.
E. Balaraman, B.A.
P. Narayanan
G.K. Loganathan, M.A.
R. Krishnamurthy, B.A.
S.C. Ramaiah
H. Dhanasekaran
K.V. Parandaman, B.A.
K. Somasekar
S. Kumar, B.Com.

Contact persons

Disease survey
Annual risk of tuberculosis infection
Field
Dr. C. Kolappan
Dr. K. Sadacharam
Mr. Nagabooshana Rao
trcicmr@md3.vsnl.net.in(admin2)

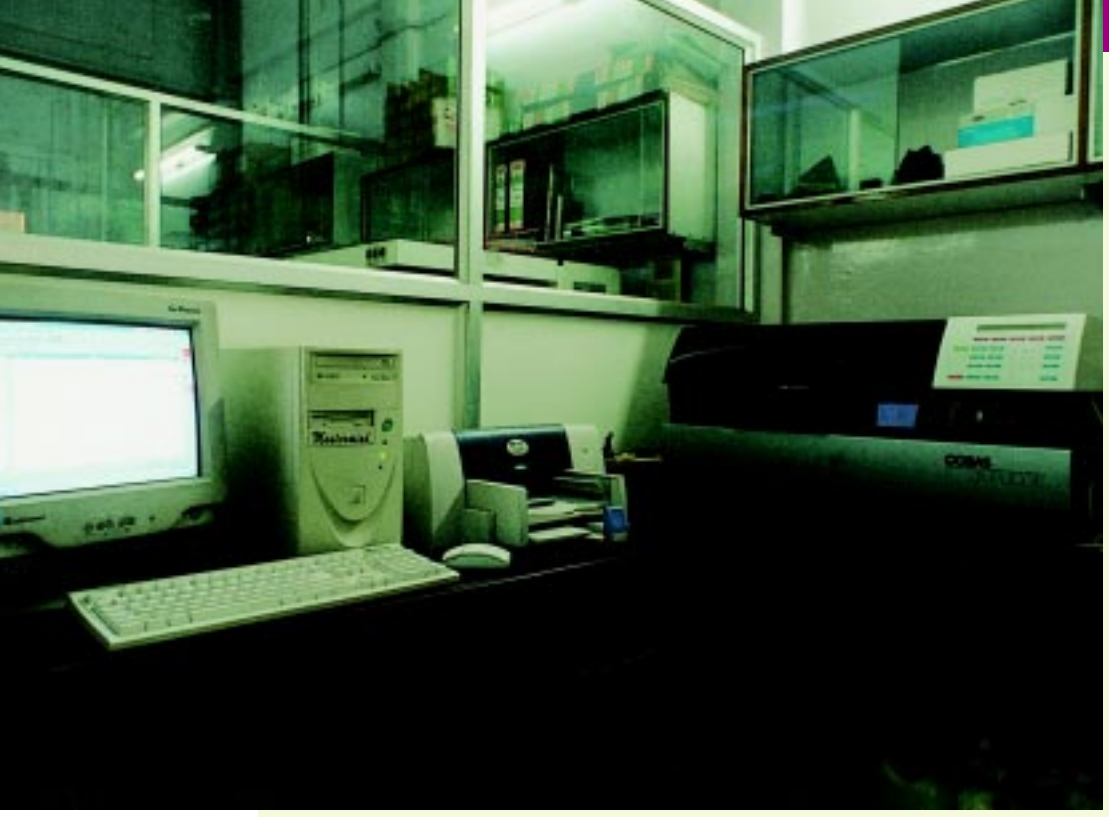
Smoking and tuberculosis
Dr. C. Kolappan
trcicmr@md3.vsnl.net.in(admin2)

Statistics
Mr. P.G. Gopi
trcicmr@md3.vsnl.net.in(epidstat)

Electronic data processing
Mr. R. Subramani
trcicmr@md3.vsnl.net.in(edpincharge)

Molecular epidemiology
Dr. Sujatha Narayanan
trcicmr@md3.vsnl.net.in(mdp5sn)

Dr. Sulochana Das
trcicmr@md3.vsnl.net.in(imm2)



HIV/AIDS

The centre is continuing its effort to focus on research evaluating the diagnostic criteria and strategies for prevention and treatment of TB in HIV-infected persons. This is being done in collaboration with the Government Hospital for Thoracic Medicine, Tambaram, Government General Hospital, Chennai and the Government Rajaji Hospital, Madurai. In addition, laboratory studies have been conducted to assess viral load and CD4 counts in HIV+ subjects at varying stages of the disease and the complex interplay between HIV and TB on the cell-mediated immune response, particularly cytokine production by lymphocytes and its correlation with the level of immunodeficiency in the patient. A study of the role of immune activation markers in assessing response to treatment and prognosis in patients with HIV and TB is also planned. Sociological studies include the quality of life in patients with HIV and TB, behavioural intervention to decrease transmission and examining HIV vaccine readiness in the community. Future plans include studies of opportunistic infection in HIV, paediatric HIV and the socio-economic impact of HIV on the individual and family. Also, studies to assess lung function impairment in patients treated for pulmonary TB and the role of inhaled steroids in ameliorating the sequelae are being undertaken.

An evaluation of diagnosis, treatment and prevention of tuberculosis in HIV-infected individuals

The aims of this study are to

- evaluate the sensitivity and specificity of the current RNTCP algorithm for diagnosis of TB among HIV-infected persons
- assess the efficacy of RNTCP regimens among HIV-infected persons with pulmonary TB or tuberculous lymphadenitis and to study any additional benefit of an extended continuation phase
- study the relationship between stage of HIV disease and response to anti-TB treatment and to study recurrent TB in detail by using RFLP
- compare the efficacy of two TB preventive therapy regimens (H 300 mg daily for three years versus E 800 mg and H 300 mg daily for six months) in reducing the incidence of TB and mortality among HIV-infected persons

The study is being conducted simultaneously in Chennai and Madurai. Intake to the study was started in January 2001.

Evaluation of RNTCP regimens

Ninety-six patients have been admitted to the study–39 into standard category I, 39 into category I with extended continuation phase (trial regimen), 13 into category II and 5 into category III. The mean age of the patients is 33 years (range 17-63); there are 74 males and 22 females. Also, 54 per cent of patients had CD4 counts <200 cells/cu.mm. Smear conversion at two months was observed in 72 per cent of patients while 91.5 per cent became sputum culture negative, indicating a satisfactory initial response to chemotherapy. Compliance has been good and patients are being followed up to assess cure and relapse rates. There have been 10 deaths so far, three due to TB and seven due to other causes.

Preventive therapy for TB in HIV-infected persons

So far, 196 HIV+ individuals have been admitted to the study and randomly allocated to the two regimens. There are 75 males and 121 females, and the mean age of the patients is 28 years (range 18-48 years). The mean body weight is 48 kg (range 30-82 kg). 50% of subjects had Mantoux test reaction >5mm. So far, there have been two cases of culture positive TB while 5 others have had anti-TB therapy instituted on clinical grounds (clinical or radiological deterioration). A five-year follow-up is planned to assess TB incidence and mortality rates in the two groups.

Residual lung function impairment in patients treated for pulmonary tuberculosis and the role of inhaled steroids: a double blind randomised controlled trial

An earlier study conducted at our centre revealed that about 50 per cent of patients who had been treated for pulmonary TB with standard short course regimens had pulmonary function abnormalities (either restrictive or obstructive type of defects). Though TB patients were bacteriologically cured of their disease, they continue to have respiratory symptoms suggestive of chronic airway obstruction. Inhaled steroids are anti-inflammatory agents that act topically with minor systemic side-effects and are widely used in the management of chronic asthma. The objectives of this study are to:

- characterise the physiological and functional changes / abnormalities in lung function that occur in patients treated for pulmonary TB; including detailed analysis of pulmonary function at rest and during exercise
- study the effect of inhaled steroids in reducing or reversing the residual defect

Details of the study methodology were given in the Annual Report 2000. Thirty-five patients cured of TB were evaluated by spirometry, lung volumes and exercise stress tests. In addition, 88 patients were admitted to the study, of which 48 have completed three months of therapy. Of them, 24 were found to have abnormal pulmonary function at three months and have been randomly allocated to either inhaled steroid or placebo groups. They will be further followed with pulmonary function tests every three months up to 24 months. Patients who have normal lung function at the end of anti-TB treatment will be evaluated again at 12 and 24 months, respectively, to look for late onset sequelae. The study is in progress.

Health-related quality of life in patients with HIV and tuberculosis

Patients with HIV and TB have a variety of health-related problems which affect their daily activities and hence their quality of life. This study aims to assess the quality of life of patients with HIV and TB at the beginning and end of TB treatment. Patients are asked about their health and emotional problems using the self-report questionnaire developed by Gordon Guyatt. A visual analog scale is used to grade the response.

Fifty patients with HIV and TB completed the questionnaire before start of anti-TB treatment and 22 after completion of therapy. A significant improvement in physical as well as emotional parameters take place at the end of treatment. Scores will also be correlated with the lung function as assessed by spirometry. It is planned to study 100 patients. The study is in progress.

Staff list

Soumya Swaminathan, M.D, Dip.N.B.
K.V. Kuppu Rao, Ph.D.
Pradeep Aravindan Menon, M.B.B.S.
Sudha Subramaniam, Ph.D.
Luke Elizabeth Hanna, M.Sc.
K. Sankaran, B.Sc., M.L.T.
Beena Elizabeth Thomas, M.A.
Mohanarani Suhadev, M.A
Meenalochani Dilip, M.A.
N. Valarmathi, B.Sc
Annamma Joy
Mary Eunice George

Contact persons

Clinical aspects of HIV/TB
Dr. Soumya Swaminathan
Dr. Pradeep Menon
trcicmr@md3.vsnl.net.in(hiv1)

Laboratory aspects of HIV/TB
Dr. Sudha Subramaniam
Mrs. Luke Elizabeth Hanna
trcicmr@md3.vsnl.net.in(hiv1)

Pulmonary function tests
Dr. K.V. Kuppu Rao
trcicmr@md3.vsnl.net.in(hiv1)

Behavioral studies in HIV/TB
Mrs. Beena Elizabeth Thomas
Mrs. Mohanarani Suhadev
Mrs. Meenalochani Dilip
trcicmr@md3.vsnl.net.in(hiv1)

Immunology

Purification of *M.tuberculosis* antigens

In the previous year's report, results of serum antibodies against the three purified antigens (38 kDa, Antigen 85 Complex (30/31 kDa) and 16 kDa) and the outcome of a combination of antigens were shown in the adult population. Here, the results of evaluation in sera samples from other "difficult-to-diagnose" categories, such as childhood TB and HIV- TB are given. Free circulating antibodies of class IgG, A and M have been measured in the above categories. The results of the three isotypes were combined and analysed.

Childhood TB

The study population consisted of 87 children below the age of 15 years. Childhood tuberculosis patients included both pulmonary and extrapulmonary forms (N=26). Out of 26 cases, nine were either smear and/or culture positive, three had TB lymphadenitis confirmed by bacteriology or histopathology. Others were clinically diagnosed. The control group comprised of normal school children of less than 15 years of age (N=61).

The number and percentage of sera positive for the three purified antigens can be seen in Table 10.

The 38 and 30 kDa showed a sensitivity of 81 and 85 per cent respectively. The 16 kDa antigen had lesser sensitivity of 73 per cent. The specificity ranged from 97-98 per cent.

Both the two smear positive cases and 4/7 culture positive cases were positive for anti 38 kDa antibody. Similar results were obtained with 30 kDa too. In addition, of the 17 cases, which were clinically categorised without bacteriological evidence, 15 cases with 38 kDa and 16 with 30 kDa were confirmed by the ELISA test.

Table 10 Antibody positivity in Childhood TB

ISOTYPE	38 kDa		30 kDa		16 kDa	
	No. +ve	% Sen.	No. +ve	% Sen.	No. +ve	% Sen.
IgG	18	69	17	65	17	65
IgA	9	35	8	31	3	12
IgM	5	19	6	23	5	19
IgG+M	20	77	19	73	17	65
IgG+A+M	21	81	22	85	19	73

When positivity for different antigens was considered, a maximum number of 22 of 26 cases (84.6 per cent) were recognised by 30 kDa antigen alone. The 38 and 16 kDa antigens did not react positively with additional sera, not recognised by 30 kDa.

Among the three antigens used in this study, the 30 kDa antigen gave the highest sensitivity and specificity. Combining the three isotypes (IgG, A & M) was found to increase the sensitivity of the assay without significantly compromising specificity. This finding may have potential practical application, since the method can be used to confirm clinically suspected cases.

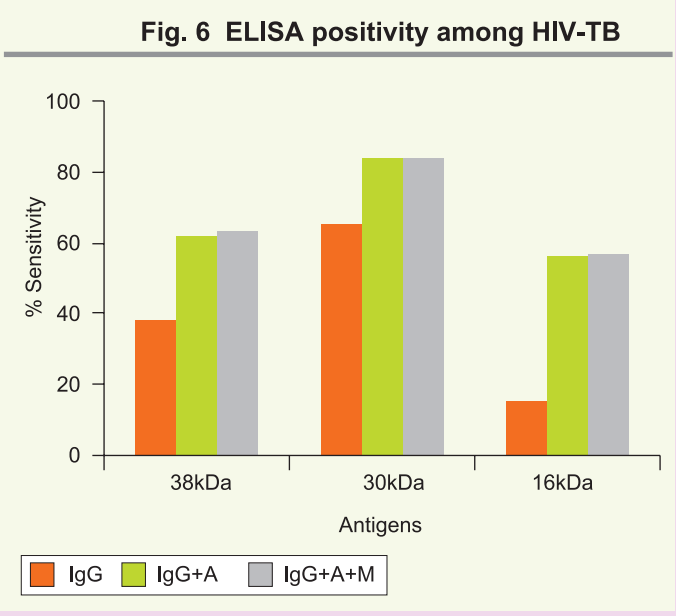
HIV-TB

The study population consisted of 393 adults. HIV seropositive TB patients were 68 in number. Of them, 13 were smear negative and 55 smear positive. In addition, 175 HIV seronegative TB patients and 150 normal healthy subjects were also included.

ELISA positivity obtained with anti-38 kDa IgG antibodies was less in HIV-TB patients, when compared to HIV seronegative TB patients (38 per cent vs 61 per cent) as shown in Figure 6.

However, in the case of IgA alone, there was increased positivity in HIV-TB. The overall results for IgG and A in HIV-TB patients was 62 per cent.

Fig. 6 ELISA positivity among HIV-TB



For 30 kDa too, the sensitivity increased to 84 per cent, upon combination of the three isotypes. Anti-16 kDa (G+A+M) detected positivity in 57 per cent. Moreover in 11 out of 13 (85 per cent) cases of HIV-TB with smear negative tuberculosis, anti-30 kDa was positive. The 38 and 16 kDa had 54 per cent and 46 per cent sensitivity, respectively, in this group (Data not shown). Patients were also categorised according to their CD4 counts and difference in their antibody response was analysed by ELISA and immunoblot. However, there was no difference in recognition in relation to CD4 counts.

The 30 kDa antigen was found to be useful in the diagnosis of both childhood TB and HIV-TB. It also proved to be of as much diagnostic utility in HIV-TB, as in HIV negative TB, irrespective of the severity of immunosuppression and smear status.

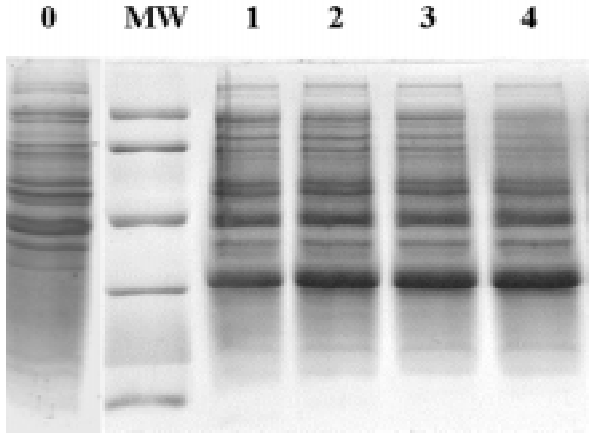
Cloning, expression and purification of 27 kDa Rv 3803c protein (MPT51) of *M.tuberculosis*

The 27 kDa (MPT51) antigen was observed in our laboratory to be specifically recognised by TB sera in immunoblots. It was thus proposed to purify the MPT51 antigen (native and recombinant) and evaluate its diagnostic value in various categories.

The antigen was prepared with culture filtrates of *M.tuberculosis* grown with continuous shaking. Purification involved hydrophobic interaction chromatography on phenyl sepharose. Both this method and subsequent attempts using preparative SDS-PAGE (whole gel eluter) yielded protein in absolute purity but not in sufficient quantity.

Attempts were therefore made to clone and overexpress the MPT51 gene in *E.coli*. The *rv3803c* gene of *M.tuberculosis* was amplified by PCR using two different sets of primers (upstream and downstream primers, corresponding to 0.8 kb fragment) with specific restriction enzyme adapters and ligated into two expression vectors, pET15b and pET24d, which contained histidine tags on the N-terminal and C-terminal regions of the gene, respectively. The insert fragments of the two constructs were verified by DNA sequencing. The plasmids that were obtained, namely, pRAMN and pRAMC were further transformed into *E.coli* BL21 (DE3) strain for expression. The recombinant gene was induced with IPTG and the expressed histidine tagged protein was further purified by Nickel NTI column as illustrated in Figure 7.

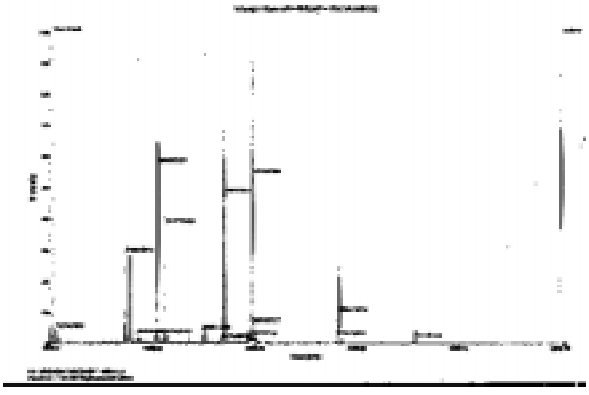
Fig. 7 SDS-PAGE showing induction of the expressed Rv3803c protein



Lane 0 : Uninduced culture
MW : Molecular weight marker
Lanes 1-4 : Induced cultures at 1,2,3 and 4 hrs

The MALDI-TOF experiment was carried out to determine the molecular mass of the proteins purified. The N-terminal and C-terminal proteins had molecular masses of 29648.28 and 28740.42 daltons, respectively. Peptide mass-fingerprinting analysis was also carried out by subjecting the protein to trypsin digestion. Figure 8 depicts the monoisotopic masses obtained for the individual peptides that were analysed and found to be in the range of 1021.456 to 4570.101. The sequences of the digested peptides were matched with the protein sequences in the database using the PROFOUND software.

Fig. 8 Mass Spectrum Analysis for the Trypsin digest of Rv 3803c by MALDI-TOF



The expressed MPT51 protein with N and C terminal histidine tag was subjected to HPLC by passing through reverse phase column (15RPC ST 4.6/100). Figure 9 depicts chromatograms of the entire run for both the proteins. A single peak was obtained for both N and C terminal proteins, which confirmed the homogeneity of these purified proteins. The chromatogram of the entire run was analysed by UNICORN V3.20 software.

The recombinant proteins so obtained will be utilised for diagnostic purpose (antibody detection) by ELISA. Besides, the cell- mediated immune response evoked by this antigen will also be studied among TB patients and normal healthy subjects by lymphocyte transformation tests and cytokine analysis. The study is continuing.

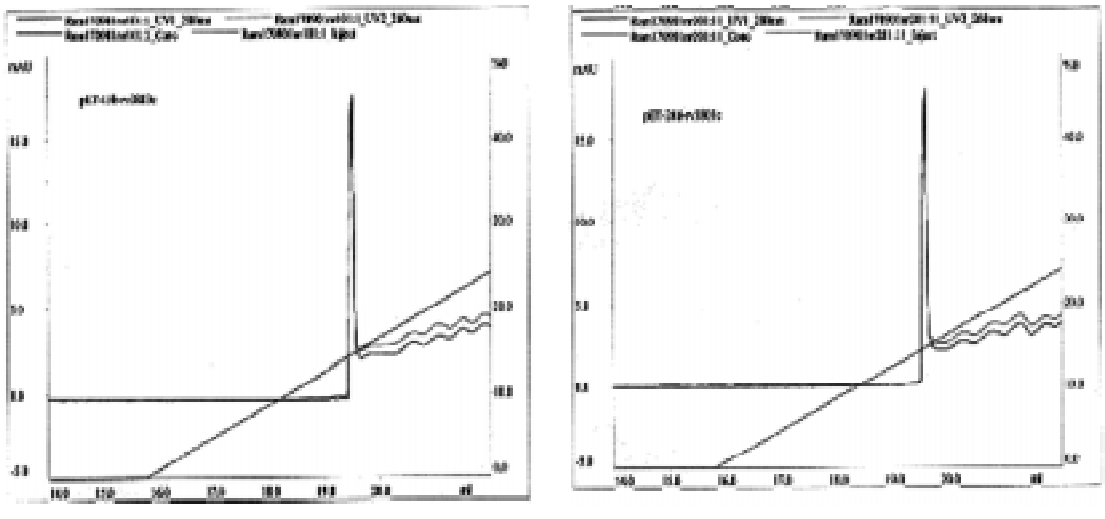
Studies on HLA and non-HLA gene polymorphisms in spinal tuberculosis

In recent years, multicandidate genes have been suggested to be associated with susceptibility to various infectious and non-infectious diseases. The main objective of the present study is to find out whether HLA and non-HLA gene variants are associated with susceptibility or resistance to spinal TB. The study was carried out in 63 spinal TB patients and 63 spouses (patient contacts).

i) HLA studies

Serological determination of HLA-A, -B, -DR (by DNA typing) and -DQ antigens was carried out in spinal TB patients and spouses. Increased antigen frequencies of HLA-A2, -A11, -B8, -B13, -B40 and -DR9 were observed in spinal TB patients when compared to controls while decreased antigen frequencies of HLA-A1 and -B5 were seen in the patient group as compared to controls. These differences were not statistically significant. However, these antigens are in strong linkage disequilibrium with other HLA antigens as haplotypes. Significantly increased haplotype frequencies of A11-B8,

Fig. 9 Chromatogram obtained from the RPHPLC studies for the purified Rv3803c protein with N and C terminal his. tags.



A11-B15, A11-B40, A11-DR3, A11-DR4, A11-DR9 and B40-DR3 were observed in spinal TB patients than in the control subjects.

The results of this study suggest that HLA antigens per se are not associated but haplotypes may be associated with susceptibility to spinal TB.

ii) Non-HLA gene polymorphism studies

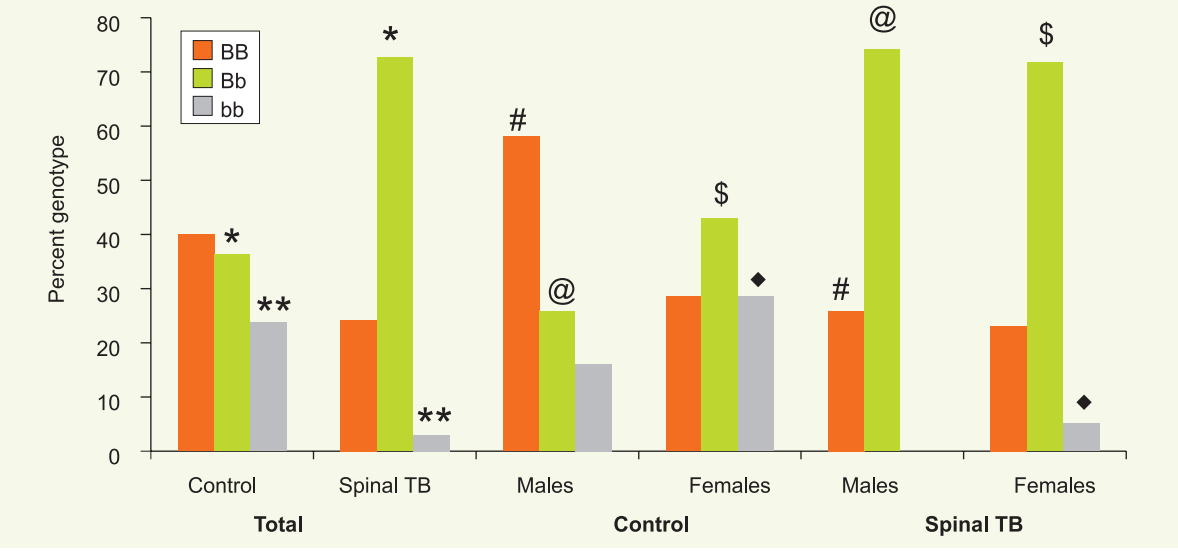
The following non-HLA gene polymorphisms (1) IL-1RA, (2) TNFa andb (3) MBL (4) NRAMP1 (5) VDR gene polymorphisms were studied in spinal TB patients and control subjects.

IL-1RA, TNFa and b, MBL and NRAMP1 gene polymorphisms did not show any association with susceptibility or resistance to spinal TB, while VDR gene polymorphic variants showed an association with spinal TB. A significantly increased frequency of the VDR genotype Bb (hetrozygotes) of *Bsm*I polymorphism was observed in the spinal TB patients when compared to the contacts. Significant increase of the variant genotype bb (homozygotes of the infrequent allele of *Bsm*I polymorphism) was seen in the contacts compared to the patients. These differences were also observed in male and female patients and contacts as seen in Figure 10.

Moreover, an increased genotype frequency of VDR genotype tt (homozygotes of the infrequent allele of *Taq*I polymorphism) was observed in female spinal TB patients than female contacts.

The study on the non-HLA gene polymorphisms suggests that VDR gene polymorphic variant Bb is associated with the susceptibility and bb with resistance to spinal TB. The present study on HLA and non-HLA gene polymorphisms revealed that multicandidate genes are associated with susceptibility to spinal TB.

Fig. 10 Genotype frequencies of *Bsm*I polymorphism of VDR gene in contacts and spinal TB patients



B – common allele; b – infrequent allele of *Bsm*I polymorphism

***P=0.00002; Odds ratio (OR) : 4.7; **P=0.00009; OR : 0.1; #P=0.028; OR : 0.25; @P=0.0007; OR : 8.2; $P=0.01; OR : 3.4; ◆P=0.01; OR : 0.14**

Non-HLA gene polymorphisms in pulmonary tuberculosis

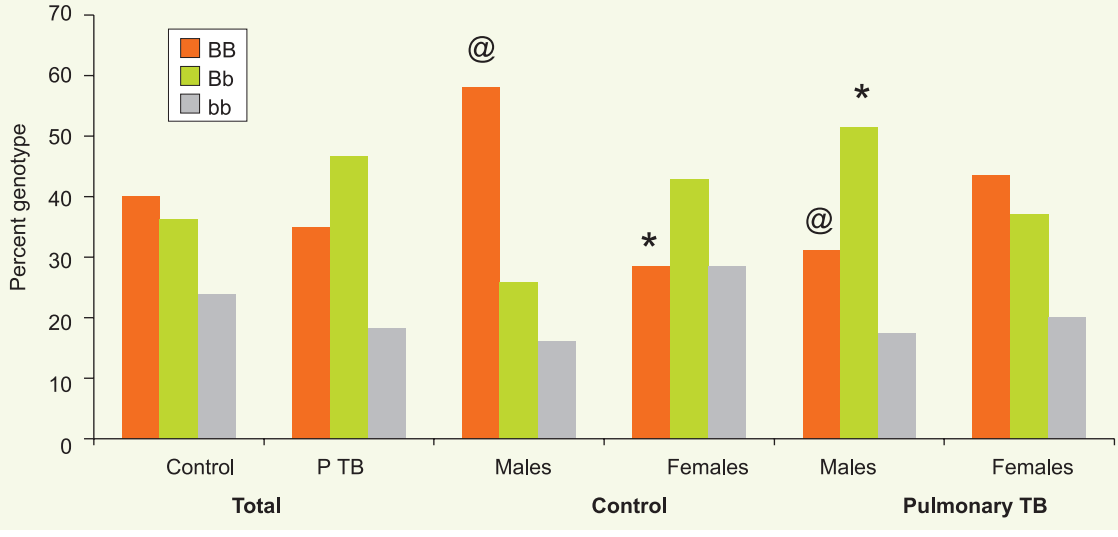
Our earlier studies in pulmonary TB revealed an association of HLA-DR2, FMH of MBL and mutant genotype tt of *Taq*I polymorphism of VDR genes with susceptibility to pulmonary TB. We have now looked into possible associations between NRAMP1 and VDR gene polymorphisms and susceptibility to pulmonary TB. The former was studied in 100 pulmonary TB patients and 112 control subjects. The study suggested that NRAMP1 gene is not associated with susceptibility or resistance to pulmonary TB.

Vitamin D receptor gene polymorphisms (*Bsm*I, *Apa*I and *Taq*I polymorphisms) were studied in 120 pulmonary TB patients and 80 spouses (contacts). No difference in the genotype frequencies of *Bsm*I, *Apa*I and *Taq*I polymorphism was observed between the total controls and total pulmonary TB patients. However, interesting observations were made between male and female patients and contacts. Significantly increased genotype frequency of Bb (heterozygotes of *Bsm*I polymorphism) was observed in male pulmonary TB patients when compared to control males. Increased genotype frequency of BB (homozygotes of common allele of *Bsm*I polymorphism) and tt (homozygotes of infrequent allele of *Taq*I polymorphism) was observed in male controls as seen in Figures 11 and 12.

The study suggests that Bb and TT (homozygotes of the common allele of *Taq*I polymorphism) genotypes are associated with susceptibility to pulmonary TB in males. Also, BB genotype of *Bsm*I polymorphism and tt genotype of *Taq*I polymorphism are associated with resistance to pulmonary TB in male subjects. Thus, tt genotype of *Taq*I polymorphism was found to be associated with susceptibility to pulmonary TB in female subjects and TT genotype with resistance in females.

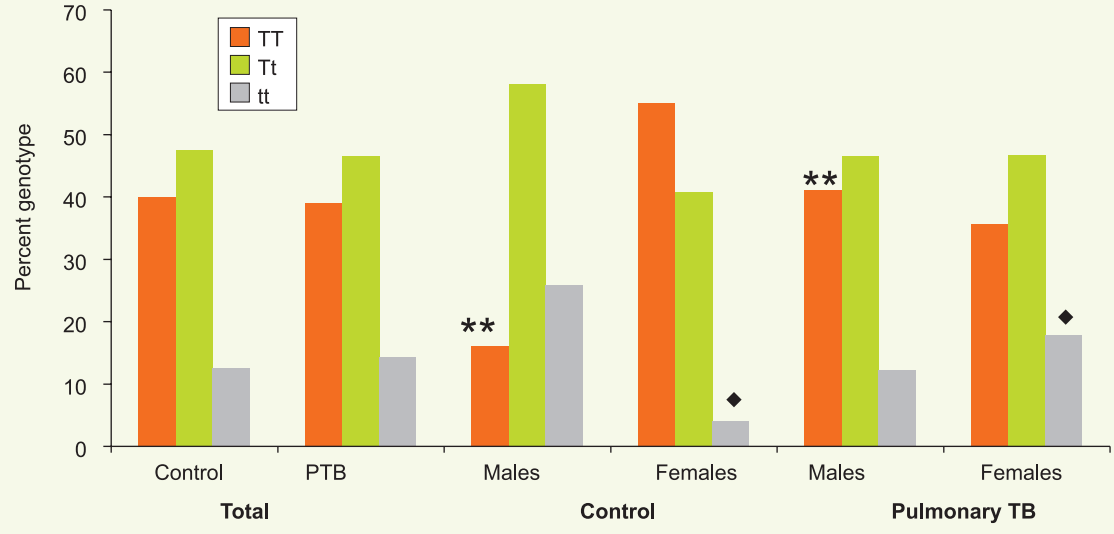
The study suggests that polymorphic variants of VDR genes are associated with susceptibility to pulmonary TB, apart from HLA-DR2 and MBL genes. Moreover, association of multicandidate genes with susceptibility to pulmonary TB is evident.

Fig. 11 VDR gene *BsmI* polymorphism in male and female controls and pulmonary TB patients



B : common allele; b : infrequent allele of *Bsm*I polymorphism
@ P=0.018; *P=0.028;

Fig. 12 VDR gene *TaqI* polymorphism in male and female controls and pulmonary TB patients



Our completed study revealed that VDR *Bsm*I, *Apa*I and *Taq*I polymorphic variants are associated with susceptibility or resistance to pulmonary TB in male and female patients. One of the VDR gene polymorphisms *Fok*I, is presently being studied. Further, *BamH*I polymorphism of CYP2D6 (human cytochrome P450 gene–encoding the drug metabolising enzyme, debrisoquine hydroxylase) gene is also being studied to find out whether variant genotypes of CYP2D6 gene are associated with bacteriological relapse of TB.

Role of HLA-DR2, mannose binding lectin, vitamin D receptor gene variants on immune functions in pulmonary tuberculosis

Our earlier studies revealed the association of HLA-DR2, FMH and VDR gene variants with susceptibility to pulmonary TB. It is known that the progression of tuberculosis is regulated/ controlled almost entirely by cell- mediated immune response of the host against pathogen. The present study has been initiated to understand the immunoregulatory role of these polymorphic gene variants on the immune mechanism of TB susceptibility. The study will be carried out in 60 pulmonary TB patients, 60 patient contacts (spouses) and 60 normal healthy volunteers. The study is in progress.

Regulatory elements involved in the expression of inducible acetamidase gene of *M.smegmatis*

Acetamidase gene of *M.smegmatis* is the first reported inducible gene of mycobacteria, which is important for the cells to utilise aliphatic amides as carbon and/or nitrogen source. More than 90-fold induction for the gene has been observed when the cells were grown in presence of an inducer like acetamide. However, the regulation of acetamidase gene expression has not been fully elucidated.

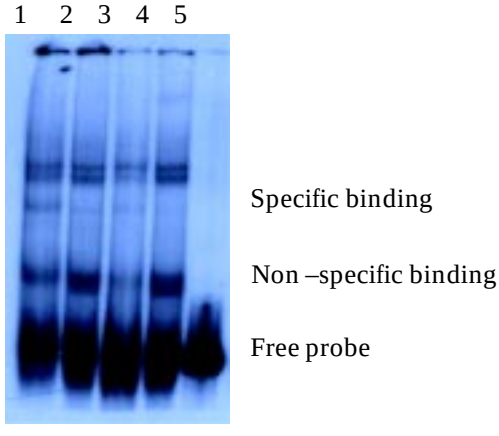
Studying the regulation of acetamidase gene expression of *M.smegmatis* will not only throw some light on our understanding on the mechanism of gene expression in mycobacteria, but also will help in constructing an inducible vector system using the acetamidase promoter for efficient expression of the mycobacterial antigen.

In our laboratory, using various molecular biological techniques such as Northern Blot Analysis, Primer Extension Analysis and RT-PCR Analysis, we have mapped the transcription start site (TSS), that lies 1.8 kb upstream from the acetamidase gene and defined minimal DNA region corresponding to acetamidase promoter. In addition, we also deciphered the mechanism of transcription for the acetamidase operon. The 4.2 kb acetamidase operon of *M. smegmatis* has been transcribed as a polycistronic message along with upstream open reading frames (ORFs) by the promoter located 1.8 kb upstream; and is processed into two transcripts; one corresponding to the upstream ORFs and the other for acetamidase.

A different upstream sequence has been cloned into a promoter probe vector pJEM13, having *lacZ* gene as reporter in order to check for the presence of regulatory regions involved in the control of acetamidase expression. A 2 kb DNA region (comprising of TSS) from the 5'-end of the acetamidase operon showed highest induction; whereas a 1 kb deletion in the acetamidase operon (between 2 kb and 3 kb) resulted in highest induction ratio (when compared with uninduced culture). In addition, removal of a 400 bp region 5' from the acetamidase operon drastically reduced the induction from the promoter.

To assess the role of the 1 kb region and the 400 bp region in the regulation of expression of acetamidase operon, these regions were radioactively labeled and subjected to electrophoretic mobility shift assay (EMSA) with induced or uninduced crude cell extract of *M.smegmatis*. To confirm the specificity of DNA-protein interaction, experiments were done by incorporating unlabeled target DNA along with the labeled counterpart and were subjected to competitive inhibition (Cold Chase). Results from the EMSA showed that specific protein are indeed binding to those DNA regions. While the 400 bp DNA fragment showed shift in mobility only when tested with cell extract from induced cells, the other fragment (420 bp fragment, 5'-to the 1 kb region) showed mobility shift with both induced and uninduced cell extracts. This shows that the 400 bp region could be involved in the gene regulation during acetamide induction and since the 420 bp region binds with protein factors from induced and uninduced cells, this DNA region could be involved in the constitutive, basal level of acetamidase expression. Figure 13 shows EMSA pattern showing specific and non-specific binding of regulatory proteins from crude extract from induced cells with the 400 bp target DNA.

Fig. 13 Mobility shift assay on amidase promoter of *M. smegmatis*

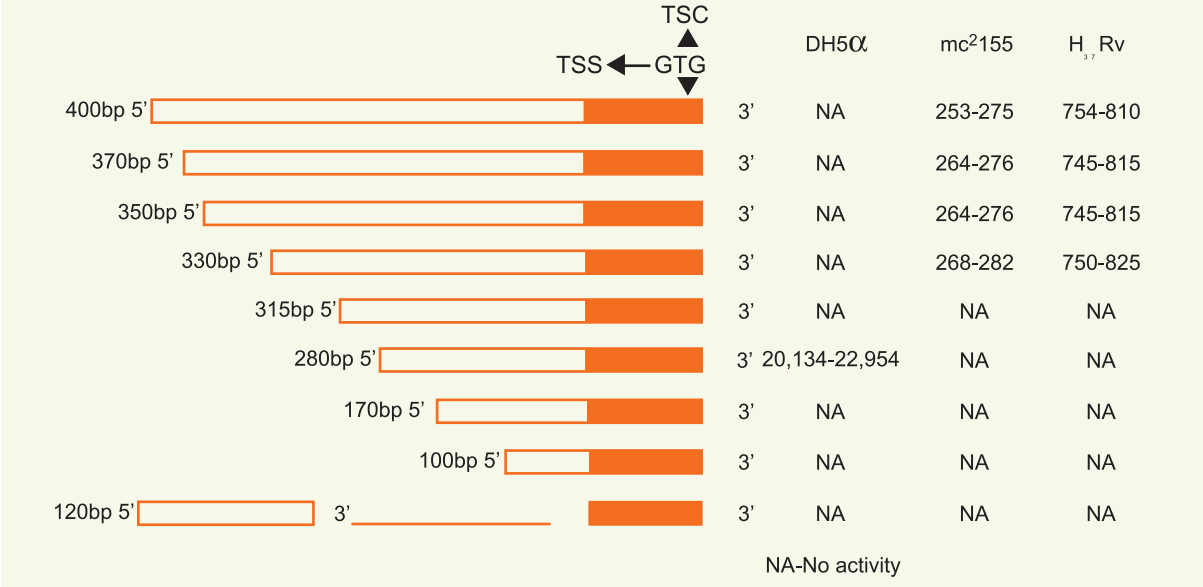


Here, 1,2,3 and 4 represent lanes in which increasing concentrations (0, 5X, 10X and 100X) of unlabeled, target region (400 bp) and fixed concentration of labeled DNA (50 ng) and crude extract (10 mg) from induced *M.smegmatis* cells has been added. Lane 5 represents the labeled probe alone.

Transcriptional analysis of *guaA* promoter of *M. tuberculosis* $H_{37}Rv$

Virulent mycobacteria must adapt to adverse conditions encountered during infectious process. This means that the amount of certain bacterial proteins must be increased in response to the changing environment while those of

Fig. 14 Deletion analysis of *gua*A promoter of *M. tuberculosis* H_{37}Rv

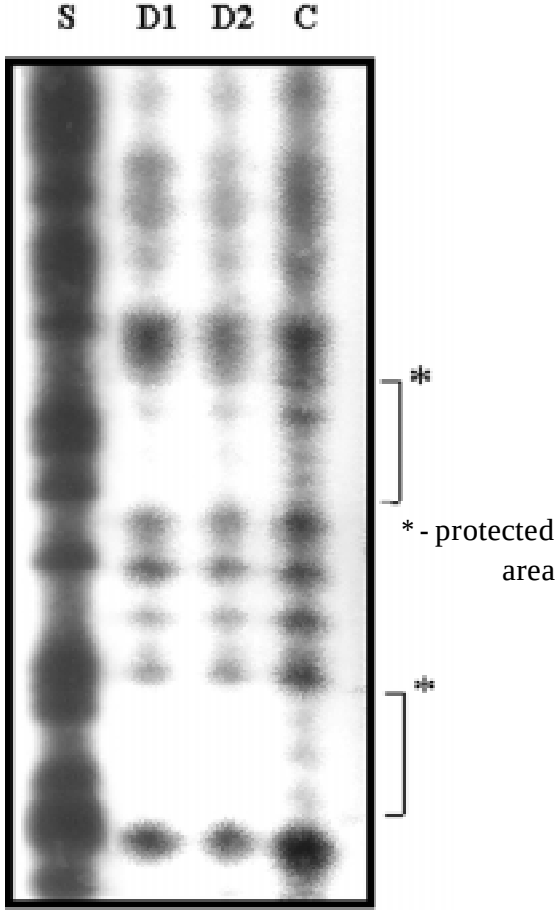


others must be lowered. Transcriptional control is the major mechanism regulating gene expression in prokaryotes. For an understanding of pathogenesis, it is important to know how the genes controlling these responses are activated and repressed. Regulation of gene expression can be studied by analysing the promoter region of the gene. One such promoter taken for analysis was a 400bp upstream region of *guaA* gene of *M.tuberculosis* H_{37}Rv encoding the purine biosynthetic enzyme, guanosine mono phosphate synthetase.

In our study, a 400bp putative promoter region upstream of the *gua*A gene was PCR amplified and cloned in a promoter probe vector, pJEM13. This 400bp *guaA* promoter (pSKV22) was found to be active only in *M. smegmatis* (mc^2155) and not in *E. coli* (DH5a) PCR mutagenesis on the 400bp fragment revealed that the 280bp fragment (pKAM2) with a 120bp deletion from the 5'-end was able to express *lacZ* 100-fold more in DH5a and not in mc^2155 as shown in Figure 14.

Further deletions in the 120bp region maintained the expression in *M.smegmatis*

Fig. 15 Footprinting analysis of *guaA* promoter



mc^{2}155 till the 330bp region and revealed the presence of the functional promoter to lie within 330bp and 280bp of the promoter.

Regulation studies performed on the two promoter clones showed that they were under stringent and growth rate dependent control. Gel retardation assays with *M.smegmatis* RNA polymerase (RNAP) were performed on both the promoter clones and a strong binding was observed in the case of 400bp while the binding was weak with the 280bp fragment, which confirmed the specificity of RNAP. Foot printing assay revealed the binding site for the RNAP to be between –7 and –38 bases upstream to GTG, which can be visualised in Figure 15.

Macrophage experiments to study the *in vivo* expression of the promoter fragments showed that the promoter had prolonged expression inside the macrophages.

Recombinant BCG based HIV-1-1 epitope delivery system

BCG is one of the oldest vaccines available. Till date, three billion people have been vaccinated with negligible incidence of adverse side-effects. It is the only live bacterial vaccine available and has excellent immunogenicity. The immune response elicited by BCG is multifaceted but predominantly cell-mediated. When administered orally, it induces strong mucosal antibody response. The above-mentioned attributes are vital components of protective immunity against HIV-1. Hence, BCG serves as an ideal candidate for delivering HIV-1 epitopes. Previous reports on recombinant BCG expressing HIV-1 envelope glycoprotein showed the toxic effect of this protein to the bacilli when hyperexpressed. Hence, in the present project, the principle neutralising determinant (PND) epitope of HIV-1 gp120 antigen alone will be grafted to the mycobacterial Cpn-10 antigen and the resulting chimera will be expressed in BCG. Mycobacterial Cpn-10 antigen was selected as a carrier since its crystal structure is known and its immunogenicity is well documented. Preliminary experiments showed that this antigen is highly conserved among various mycobacterial species (PCR and Southern blotting experiments were carried out using several mycobacterial genomic DNA samples). As a first step in this project, the Cpn-10 gene has been cloned in pCR2.1 and sequenced. Experiments are being carried out to graft the HIV-1 epitope. The resulting chimeric gene will be expressed in BCG and the recombinant BCG will be evaluated for its immunogenicity in a murine model.

Studies on dual signal hypothesis and apoptosis

In our previous studies, we showed that apoptotic pathways operate during active *M.tuberculosis* infection and may contribute to deletion of *M.tuberculosis* reactive T cells and the immunopathogenesis of this disease. We have also shown that high dosage of antigen and mitogen induced apoptosis and inhibited proliferation to varying degree in different tissues of mouse model.

To further investigate the mycobacterial antigen induced apoptosis, *in vitro* experiments were designed to test newer sonicate antigens prepared from the clinical isolates for its ability to induce apoptosis in normal peripheral blood mononuclear cells. Simultaneously, we estimated TNFa levels to correlate with the induced apoptosis. The results are being analysed.

Molecular and immunological characterisation of *M. tuberculosis* strains with single copy of IS6110

The insertion sequence IS6110 is widely used as a DNA fingerprinting probe for the classification of *M.tuberculosis* strains based on its copy numbers and its insertion sites on the genome. Among the low copy number strains of *M. tuberculosis*, the strain with single IS6110 copy has been reported from various geographical regions of the world. However,

the spread of this strain was only 4–5 per cent in these regions. In our previous studies, we have reported *M.tuberculosis* strains with single IS6110 copy in south India. The percentage spread of this strain in the community was very high, i.e. almost 40 per cent and hence a most prevalent strain in this area. Hence, protein profiles of these strains were carried out.

Sonicate antigens were prepared from 13 clinical isolates of *M.tuberculosis*. These strains had single copy of IS6110 at various insertion sites on the genome by RFLP studies. The strain S1 had IS6110 insertion at 4.5 kb region, while S2 and S3 had insertion at 1.0 kb region and the remaining S4 to S13 strains had common insertion site of 1.5 kb in *M.tuberculosis* genome. The protein profiles of these strains were compared with the protein profile of standard strain *M.tuberculosis* $H_{37}Rv$ on 10 per cent polyacrylamide gel and the results were analysed.

The results showed differential expression of many proteins. In high molecular weight region, there was not much difference in protein levels. However, in low molecular weight regions, many strains showed over- expression of certain proteins.

In particular, two strains (S9 & S12) showed over-expression of two proteins of molecular sizes 15 and 22 kDa. In addition, strains S7, S9 and S11 showed over-expression of 28, 29 and 13 kDa proteins, respectively. The strains S1, S2 and S3 did not show distinct variations in expression levels of most of the proteins, except 23 kDa in S2 and 18 kDa in S1.

The sonicate antigens which showed distinct protein profiles were selected for further immunological characterisation. The antigen recognition patterns of these strains with TB and non-tuberculous sera were done by ELISA and Western blot. The results are being analysed.

Staff list

V. Kumaraswami, M.D., M.N.A.M.S., Ph.D(Med)
Alamelu Raja, Ph.D.
Sujatha Narayanan, Ph.D., C.T.(ASCP)
P. Selvaraj, Ph.D.
A. Ravoof, B.Sc.
Sulochana Das, Ph.D.
S. Ramanujam, B.Sc., D.M.L.T.
S. Selvakumar, M.Sc.

Contact persons

Immunology and chemotherapy of Lymphatic Filariasis
Tuberculosis and helminth co-infection
Geographical information system
Dr. V. Kumaraswami
trcicmr@md3.vsnl.net.in(imm1)

Immunodiagnosis of tuberculosis
Dr. Alamelu Raja
trcicmr@md3.vsnl.net.in(mdp4ar)

Molecular biology of *M.tuberculosis*
Molecular epidemiology of tuberculosis
Dr. Sujatha Narayanan
trcicmr@md3.vsnl.net.in(mdp3sn)

Immunogenetics of tuberculosis
Dr. P. Selvaraj
trcicmr@md3.vsnl.net.in(imm2)

Cellular immunology
Molecular epidemiology of tuberculosis
Dr. Sulochana Das
trcicmr@md3.vsnl.net.in(imm2)

Bacteriology

Simple direct sensitivity tests for early detection of resistance in *M. tuberculosis*

Detection of drug resistance in tubercle bacilli assumes importance in the present context of RNTCP. Two methods for direct sensitivity test from smear positive sputum specimens that would yield early results have already been established at this centre. In the present comparative study, based on 175 samples, the direct test by the swab culture and the concentration culture methods were performed for the drugs, H, R and E under routine conditions in order to test the robustness of the definitions recommended earlier. Direct tests by both methods for E and direct test by the swab method for R were carried out for the first time. The direct tests were compared mutually and against the established standard indirect method. Results showed that 60–70 per cent of the cultures could be classified by the second week after setting up the culture with agreement of classification with the indirect test, in the range of 92–100 per cent. More than 95 per cent of the samples yielded results by the fourth week and 82–88 per cent of cultures resistant to H and R were detected by the fourth week. The direct tests were less satisfactory for E compared with the indirect test, which by itself has often been inconsistent in most places.

Direct test from the sputum concentrated by the Petroff's method yielded results for more samples at two weeks when compared to the direct swab sensitivity test. However, this difference was not observed from the third week. For laboratories equipped to perform culture by Petroff's method, the direct test from sputum deposits yields results from the second week. Hence the advantage is that one saves four weeks delay for results. For laboratories with limited facilities, the swab culture and sensitivity method provide a feasible and reliable alternative to detect resistance within

three weeks. Besides, the method requires no special equipment like a centrifuge or costly reagents, the only decontaminant used being one per cent cetrimide solution. The LJ medium used in all the three methods is now available commercially, though it may be more economical to prepare them in a central laboratory. The definitions used in the direct test for classifying culture as sensitive or resistant are found to be reliable and simple enough for interpretation by technicians in a routine laboratory set up.

Table 11 gives a comparison of classification of susceptibility test results at three weeks based on the direct test from sputum concentrate (DC) and by the swab culture method (DS), with the classifications based on the indirect test.

Table 11 Comparison of susceptibility results between direct and indirect tests

Indirect test	Direct test	Direct tests read at 3 weeks					
		H		R		E	
		DC	DS	DC	DS	DC	DS
Sensitive	Sensitive	50	70	65	81	62	88
Resistant	Resistant	27	38	21	25	6	11
Agreement		99	97	99	95	89	90
No. of tests		89	130	101	129	89	129
Results available		78	111	87	111	76	110
%		88	85	86	86	85	85

The comparative study between swab culture and sputum concentrate methods provide an excellent tool for those looking for simple methods of early detection of drug resistance in tubercle bacilli.

Comparison of genotypic and phenotypic assays for the early identification of multidrug-resistant *M.tuberculosis*

Rapid detection of R resistance in *M.tuberculosis* enables early identification of MDR TB, as R resistance serves as a surrogate marker. A new, rapid method, viz., DNA-Lanthanide fluorescence spectroscopy was developed, evaluated and compared with four other rapid methods (2 genotypic and 2 phenotypic) using 201 *M. tuberculosis* isolates (101 R^r and 100 R^s). The genotypic assays include: DNA sequencing, PCR-Single Strand Conformation Polymorphism (PCR-SSCP). The phenotypic assays include: LRP assay and Phage amplified biological assay (PhaB). The sensitivity and specificity of these assays were compared with the conventional indirect sensitivity test (gold standard) (Table 12).

Table 12 Performance of various assays with respect to conventional indirect sensitivity test

Assay	Sensitivity	Specificity	PPV	NPV	KAPPA	Time Required (Hrs)*
DNA sequencing	97%	100%	100%	97%	0.97	24
PCR-SSCP analysis	76%	100%	100%	81%	0.76	24
DNA-Lanthanide analysis	88%	86%	86%	88%	0.74	48
LRP assay	86%	78%	79%	85%	0.63	76
PhaB assay	97%	84%	86%	97%	0.81	78

PPV – Positive predictive value
NPV – Negative predictive value
*- Time required after isolation of primary culture

It was found that DNA sequencing gave the maximum sensitivity and specificity with the least turnaround time (24 hours).

Activity of metronidazole alone and in combination with H and R against dormant or semidormant *M.tuberculosis* in murine model

The study design and the treatment protocol of this experiment have been described in the Annual Report 2000. The results of the animal experiments pertaining to spleen and lung are given in Table 13. The total number of animals in each group was 16. The number of animals that died during treatment is given in brackets.

The results of this experiment showed no additive or bactericidal activity of metronidazole alone or in combination with H, R and HR. This may perhaps be due to the treatment protocol, which included treatment with HRZ for the initial two months period, or the inherent limitation of the mouse model employed.

Table 13 *In vivo* **activity of metronidazole**

Group	(a) Spleen			(b) Lung		
	Number of mice positive for *M.tuberculosis*			Number of mice positive for *M.tuberculosis*		
	At the end of treatment (6 months)		3 months after stopping treatment	At the end of treatment (6 months)		3 months after stopping treatment
	HCA	No HCA		HCA	No HCA	
Control	13	1	5 (11)	15	10	4 (11)
M	7 (7)	12	1	9 (7)	14	15
HM	12 (3)	0	4	13 (3)	1	5
RM	9	6	1	13	9	0
HR	0	0	3	2	0	2
HRM	2 (1)	0	4	4 (1)	0	2

§ HCA – Hydrocortisone acetate
§ M - Metronidazole

Drug susceptibility testing of clinical isolates of *M.tuberculosis* for S, H, R and E by LRP assay

The aim of this study was to find out the agreement between drug susceptibility testing of clinical isolates of *M.tuberculosis* by the conventional method and direct LRP-85 assay for the four anti-TB drugs.

In our earlier work, we compared the regular LRP assay and a rapid three-day assay named direct LRP-85 with the conventional drug susceptibility testing of *M. tuberculosis* isolates using two phage constructs, phAE129 (D29-based) and phAE85 (TM-based). Direct LRP-85 assay, using phAE85 and an inhibition index of 50 per cent, showed an agreement of 95 per cent compared to conventional drug susceptibility results both for H (1µg/ml) and R (2µg/ml). The assay was further extended to the other two important anti-TB drugs namely, S and E at two different concentrations viz.2 and 6µg/ml.

Using direct LRP-85, 222 *M.tuberculosis* clinical isolates were tested for drug sensitivity to all the four anti-TB drugs and the results were compared with the conventional sensitivity test. The results with H (1µg/ml) and R (2µg/ml) were comparable with our previous findings. Streptomycin (2µg/ml) showed a sensitivity of 77 per cent and specificity of 92%. With E, at 6µg/ml, 83 per cent of resistant strains were picked up as resistant, while specificity was poor (87 per cent).

Evaluation of Fast plaque® TB assay kit-II

Although Fast plaque® TB assay done with sputum deposits on the day of processing (Annual Report 2000) alleviated the problem of contamination, it did not compare well with conventional smear and culture procedures.

In order to improve the performance of the assay, about 100 sputum samples processed according to Petroff's procedure were subjected to the action of antibiotic supplement, incubated overnight and then processed by Fast plaque® TB assay. The results were compared with those obtained by fluorescence staining and culture on LJ slopes and are given in Tables 14 and 15.

Table 14 Comparison of results between Fluorescence smear and Fast plaque kit

Fast plaque®	Smear				Total
	+++&++	+	Total Positives	Negative	
>300 plaques	17	15	32	1	33
20 to 300 plaques	4	13	17	14	31
Negative	1	7	8	28	36
Total	22	35	57	43	100

Table 15 Comparison of results between culture and Fast plaque kit

Fast plaque®	Smear				Total
	+++&++	+	Total Positives	Negative	
>300 plaques	30	2	32	1	33
20 to 300 plaques	12	6	18	6	24
Negative	0	11	11	31	42
Total	42	19	61	38	99

The Fast plaque TB assay showed improved specificity and sensitivity compared to conventional smear and culture procedures.

Studies on drug resistance surveillance in four districts

One of the recommendations of the expert group meeting on drug resistance organised by the Central TB Division at the centre in September 1997 was that a systematic ongoing surveillance of drug resistance in newly diagnosed TB patients should be undertaken on a continuous basis in order to provide information on programme performance. It was decided that TRC would conduct such studies at North Arcot (Tamil Nadu), Raichur (Karnataka), Wardha (Maharashtra) and Jabalpur (Madhya Pradesh). These districts were chosen because baseline information was already available and a resurvey would enable an understanding of the trend in resistance over the years.

Accordingly, surveys in the two districts of North Arcot and Raichur were completed and the results presented in the previous year's report.

The study in Wardha district was started in August 2000. A total of 302 patients have been admitted and the bacteriological findings are shown in Table 16.

Table 16 Surveillance in drug resistance in tuberculosis in Wardha district, Maharashtra 2000-01

	History of previous Rx			
	Primary Resistance		Acquired Resistance	
	N	%	N	%
Total tested	197		9	
Fully sensitive	158	80	2	22
Any resistance	39	20	7	78
Any H resistance	30	15	7	78
Any R resistance	1	0.5	7	78
Any HR resistance	1	0.5	7	78

Quality assurance studies in sputum smear microscopy in eight state TB laboratories

Sputum smear microscopy is a key component of the DOT strategy in RNTCP both for diagnosis as well as follow up of the patients. Reliable laboratory results are essential for proper diagnosis, categorisation of the patients, decision to start the continuation phase and declare the patient as cured. Standardisation of the technique for microscopic examination of sputum smear has become a necessity in order to organise the existing laboratories into an inter-related network and thus obtain comparable results, facilitating the evaluation of performance and increase efficiency. With this objective in view, the centre undertook a proficiency testing programme at eight state level TB centres at six-monthly intervals. Five rounds of testing have been completed so far, the major findings of which are reported here.

Participants: The laboratories which participated in this study included the state TB demonstration and training centres at Bhopal (Madhya Pradesh), Chennai (Tamil Nadu), Cuttack (Orissa), Darbhanga (Bihar), Hyderabad (Andhra Pradesh), Nagpur (Maharashtra), Patna (Bihar) and Thiruvananthapuram (Kerala).

Slides: A panel consisting of 100 stained smears (Rounds I-V) and 50 smears (Round V) were coded and sent by post to the participating laboratories for examination and the reports were received and analysed.

Positive consistency: The proportion of positive consistency for all the five rounds centre-wise showed an overall range of 38-100 per cent indicating considerable under- reading in some centres.

Negative consistency: The agreement with respect to negative smears illustrated that majority of readers from all the centres in all five rounds had yielded 100 per cent

consistency, indicating the absence of gross over-reading of this grade.

Overall agreement: An agreement of over 95 per cent was seen with Bhopal, and above 90 per cent in Chennai, Cuttack, Hyderabad (except round IV), Nagpur, Patna and Thiruvananthapuram (except round IV). The centre at Darbhanga was consistently a poor performer (except in round II).

Isolation and characterisation of new environmental mycobacteriophages to improve the sensitivity of LRP assay

Luciferase reporter phage assay is highly specific but less sensitive. Thus, there is a felt need to look for new mycobacteriophages from environmental samples, in order to develop LRP constructs of higher sensitivity compared to the ones already developed from TM4 and D29 phages. Accordingly, eight new mycobacteriophages were isolated from soil samples collected near chicken coops, cow sheds, tuberculosis clinics and from the banks of Couum river in Chennai. Three of these phages were lytic (Che3, Che7 and Che11) and the other five were lysogenic (Che6, 8, 9,10 and 12).

These phages had varying host ranges. Mycobacteriophage Che3 could infect only *M.avium* mc^{2}2501 while Che7 was found to infect *M.tuberculosis* and *M.bovis* BCG. Che11 and a lysogenic phage termed Che12 infected *M.tuberculosis*. None of the other phages (all lysogenic) were able to infect *M.tuberculosis*, *M.bovis* BCG or *M.avium* mc^{2}2501.

Restriction enzyme digestion and gel electrophoresis showed unique bands with Che3 and Che9 phages as compared to D29 and TM4. DNA prepared from Che3 and Che9 has been sent for sequencing to Graham Hatful's laboratory at the University of Pittsburg. Electron microscopic examination of the Che3 DNA showed only one type of phage particle while the lysogenic phage Che9 showed three different types of phage particle. Lysogens of one phage are immune to super-infection by the same phage. Lysogens are the host cells loaded with lysogenic phage particles. In order to know whether the new lysogenic phages are different from one another, lysogens of Che9, Che10, L5 and BXZ2 in the top agar were spotted with dilutions of all the four phages. The new phages were found to be different from one another.

Ongoing studies

- Analysis of molecular basis of fluoroquinolone resistance in mycobacteria
- Study on the presence of inteins in essential genes, *recA* and *gyrA* in *M. tuberculosis*
- Antituberculosis activity of clofazimine

Staff list

C.N. Paramasivan, Ph.D., D.Sc., FAMS.
N. Selvakumar, Ph.D.
Vanaja Kumar, Ph.D.
Ranjani Ramachandran, M.D.
Lalitha Hari, M.Sc.
M. Naseema, M.Sc., Ph.D.
K.J. Illampurnam, B.Sc.
K. Sudhamathy, B.Sc., D.M.L.T.
Nalini Sundar Mohan, B.Sc.
Dakshayani Govindhan, B.Sc.
G. Kubendiran, Ph.D.
A. Shyam Sundar
S. Jagadeesan
P. Jaganathan
N. Saradha, B.Sc., D.M.L.T.
Samuvel Vasanthan Goodwill, B.Sc., B.Ed., D.M.L.T.
S. Manoharan, B.Sc., D.M.L.T.
Sulochana Somasundaram, M.S.(MLT.), D.M.L.T.
Daisy Vanitha, M.Sc.
K. Rajagopal, B.Sc., D.M.L.T.

Contact persons

Drug resistance surveillance
Quality control and quality assurance
Early bactericidal activity
Dormancy
Environmental mycobacteria
Dr. C.N. Paramasivan
trcicmr@md3.vsnl.net.in(bact3)

Training programmes
Early diagnosis of tuberculosis
Molecular mechanisms of rifampicin resistance
DOTS project
Dr. N. Selvakumar
trcicmr@md3.vsnl.net.in(bact2)

Luciferase phage reporter assay
Development of newer phage reporter systems
Evaluation of diagnostics
Dr. Vanaja Kumar
trcicmr@md3.vsnl.net.in(bact4)

Opportunistic infections – HIV/AIDS
Dr. Ranjani Ramachandran
trcicmr@md3.vsnl.net.in(bact1)

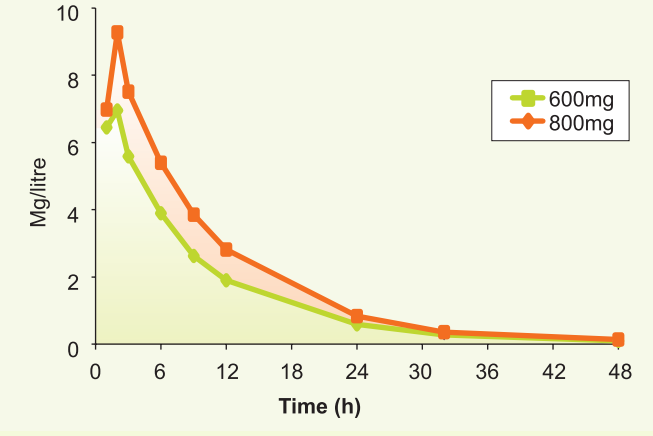
Luciferase phage reporter assay
Development of newer phage reporter systems
Dr. K. Jayashankar
trcicmr@md3.vsnl.net.in(bact4)

Biochemistry

Dose related pharmacokinetics of ofloxacin in healthy volunteers

A study regimen of 3-5 months duration employing H, R, Z and a powerful bactericidal drug, namely O, daily for treatment of pulmonary TB undertaken at our centre showed encouraging results. Currently, the efficacy of intermittent regimens using O is being tested at our centre. Ofloxacin being a concentration dependent drug, it is essential to get information on peak levels to see the ratio between MIC and peak concentration (C_{max}). Hence, an investigation was carried out by seven healthy volunteers to obtain this information following administration of 600 and 800 mg of O. Ofloxacin levels were determined in plasma and saliva at 1,2,3,6,9,12, 24,32 and 48 hours and in urine collected over a period of 24 hours by HPLC. The mean plasma of concentrations of O are depicted in Figure 16. The pharmacokinetic parameters were calculated on the basis of non-compartmental model using WinNonlin software.

Fig. 16 Mean O concentrations in plasma after 600 and 800 mg



An increase of 22 per cent in C_{max} and 40 per cent in area under the

The higher dose of 800 mg resulted in significantly higher concentrations at all the time points, except at 1 hr. ($p<0.05$). The mean trough levels for thrice weekly dosage (48 hrs) were 0.1 mg/litre for 600 and 0.13 mg/litre for 800 mg doses.

time concentration curve (AUC) were observed with higher dose. The other parameters, namely, time to attain C_{max}, half-life, the apparent volume of distribution, plasma and renal clearance and percentage of dose excreted in urine over 24 hours were independent of doses.

The ratio of O concentrations in saliva to plasma after 600 and 800 mg ranged from 0.4-0.6, which was similar for both the doses. The correlation coefficient between plasma and saliva concentrations was 0.94, suggesting that determination of O in saliva seems to be suitable for therapeutic drug monitoring.

The AUC that was calculated from concentration time curve provides an integral measure of drug exposed and therefore, an individual indicator of therapeutic value of the drug. The AUC calculated on the basis of body weight is presented in Table 17.

Table 17 AUC based on body weight

Body Weight (kg)	AUC_{24} (mg.h/litre)	
	600 mg	800 mg
40-50 (n=2)	71	107
>51 (n=5)	57	77

Subjects weighing less than 50 kg when given 600 mg dose attained AUC similar to that of 800 mg given to the subjects weighing greater than 50 kg.

The present study suggests that the dosage for intermittent therapy may be adjusted according to the body weight.

Determination of rifampicin and desacetyl rifampicin in plasma and urine by HPLC

We developed a HPLC method for the determination of R and its metabolite, desacetylrifampicin (DR) in plasma and urine, suitable for bioavailability studies using a Shimadzu HPLC system that was procured by our department recently.

The plasma method involved protein precipitation with acetonitrile, concentrating the content by vacuum drying and reconstituting with mobile phase followed by reverse-phase chromatography using RP_{18} column (250 x 4.6mm, Phenomenex Luna) and using a UV detector set at a wavelength of 254nm. The mobile phase consisted of acetonitrile: 0.05M phosphate buffer (45:55) and the internal standard was rifapentine. The retention times for DR and R were 2.9 and 4.8 minutes, respectively. The assay

was linear from 0.25 to 15.0mg/litre. The limits of quantitation and detection were 0.25 and 0.1mg/litre, respectively.

The determination of urinary R and DR is a combination of chloroform extraction and separation of the compounds by HPLC. The assay was linear from 2.5 to 80.0 mg/litre. The limits of quantitation and detection were 2.5 and 1.0 mg/litre, respectively.

Intra- and inter- day accuracy and precision data showed good reproducibility and the recovery was greater than 90 per cent for both plasma and urine assays. The representative chromatograms are given in Figure 17.

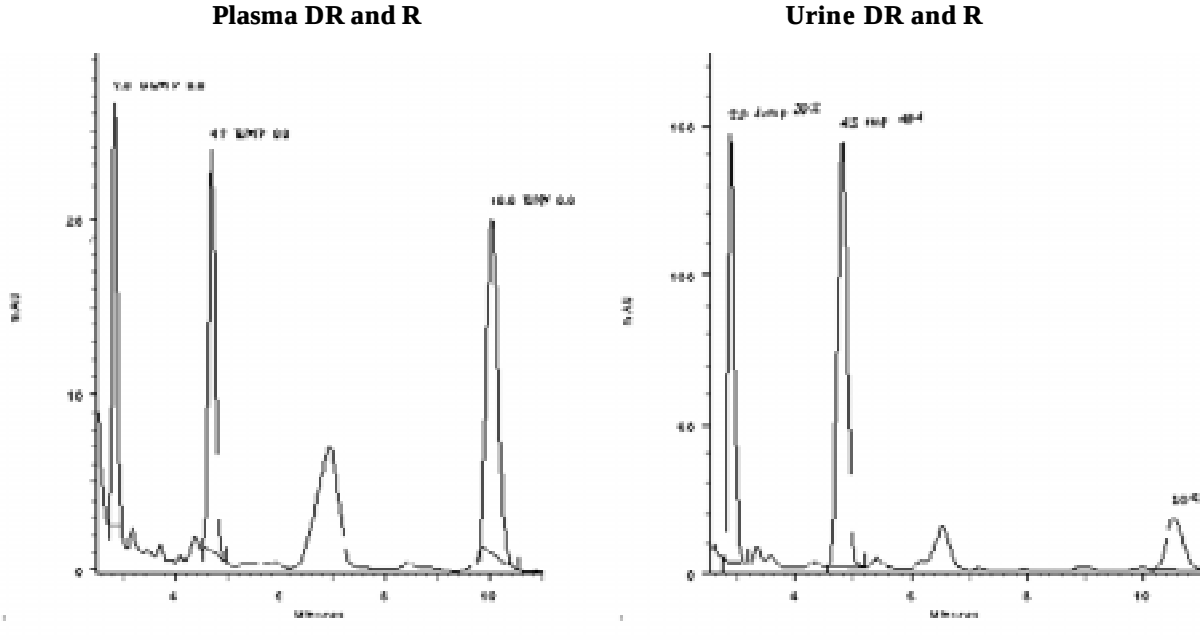
The proposed assay permits determination of R and DR separately in plasma and urine, and is useful for bioavailability, therapeutic drug monitoring and drug-drug interaction studies. The method is specific and none of the other anti-TB drugs interfere in the estimation.

Standardisation of a method for determination of ethambutol in urine and pharmaceutical preparations

A simple method for the quantitation of E in the presence of other anti-tuberculosis drugs, namely R, H and Z in fixed dose combinations (FDC), and also to quantify E in urine in the presence of other anti-tuberculosis drugs for pharmacokinetic studies was devised. The method involves extraction with chloroform to remove R and passing the aqueous phase through Amberlite IRC-50 to eliminate the other interfering anti-TB drugs. Ethambutol was then reacted with an ammoniacal copper solution and read at 290 nm.

The assay was specific for the estimation of E in urine and pharmaceutical preparations with linearity from 50 to 400 µg/ml. The sensitivity of the method was approximately 25 µg/ml. The recovery of E was 90–95 per cent from urine containing all the other anti-TB drugs. The drug was found to be stable in urine for three days.

Fig. 17 Chromatograms from HPLC analysis of R & DR in plasma and urine



A specific method for estimation of E in urine and pharmaceutical preparations has been standardised. This method can be applied to test the bioavailability of the drug in FDCs and in pharmacokinetic studies.

Ongoing studies

Screening of traditional medicinal plant products for anti-mycobacterial activity are been carried out. *In vitro* experiments using extracts from Piper longum are being tested.

Sub-therapeutic levels of anti-TB drugs in patients with HIV/TB could be due to malabsorption. Bioavailability studies of anti-TB drugs in this population will be undertaken to investigate this aspect.

Also lipid profile studies in patients with HIV/TB and Diabetes/TB are being carried out to see for possible disturbances in lipid metabolism and correlate the findings with severity of the disease.

Staff list

Prema Gurumurthy, Ph.D.
M. Kannapiran, Ph.D.
Chandra Immanuel, M.Sc.
K. Jayashankar, Ph.D.
Lalitha Victor, M.Sc.
Geetha Ramachandran, Ph.D.
K. Silambhuchelvi, M.Sc.,
A.K. Hemanth Kumar, M.Sc., D.M.L.T.

Contact persons

Bioavailability of fixed dose combinations
Pharmacokinetics of anti-tuberculosis drugs
Assay of anti-tuberculosis drugs
Dr. Prema Gurumurthy
trcicmr@md3.vsnl.net.in(biochemistry)

Clinical biochemistry
Traditional plants and drugs
Trace element metabolism
Dr. M. Kannapiran
trcicmr@md3.vsnl.net.in(biochemlab)

HPLC
Nutritional biochemistry
Mrs. Chandra Immanuel
trcicmr@md3.vsnl.net.in(biochemistry)

Luciferase phage reporter assay
Development of newer phage reporter systems
Dr. K. Jayashankar
trcicmr@md3.vsnl.net.in(bact4)

Bioavailability of fixed dose combinations
Pharmacokinetics of anti-tuberculosis drugs
Dr. Geetha Ramachandran
trcicmr@md3.vsnl.net.in(mdp5scr1)

Mr. A.K. Hemanth Kumar
trcicmr@md3.vsnl.net.in(biochemistry)

Pathology

Immunomodulatory effects of immune complexes in a guinea pig model for post-primary tuberculosis

Guinea pigs which had been sensitised with a low dose of *M.tuberculosis* were treated with immune complexes containing *M.tuberculosis* and antibody in various proportions and then challenged with live *M.tuberculosis*. The animals were euthanised at various time points from the first to the sixteenth week post-infection. Viable counts of *M.tuberculosis* in the spleen, histopathology of the spleen, liver and lung and cytological analysis of bronchoalveolar lavage were carried out. Experiments are in progress.

Protective efficacy of recombinant BCG strains in guinea pig tuberculosis

Recombinant strains of BCG over expressing esat-6, 19 kDa, 38 kDa, 85A, 85B and 85C antigens were injected into guinea pigs intradermally and nine weeks later, the animals were challenged with *M.tuberculosis* H_{37}Rv ($10X^2$ or $10X^5$) subcutaneously. These animals were euthanised either three or eight weeks post- infection and the following parameters were assessed: viable counts of *M.tuberculosis* from the spleen, histology of the liver and lung, and cytology of bronchoalveolar lavage. Experiments are in progress.

Infective potential of tyrosine phosphatase knock out strain of *M. tuberculosis*

The genome of *M. tuberculosis* has been shown to encode two putative tyrosine phosphatase genes (mptpA and mptpB). It has been hypothesised that these genes interfere in host-cell signalling pathways, thus aiding in the survival of *M.tuberculosis* in the host environment of the macrophage. These genes have been implicated in the virulence of *M.tuberculosis*. Strains of *M.tuberculosis* in which these genes have been disrupted, separately, were constructed and used for infecting the guinea pigs subcutaneously.

One group of animals was infected with the wild type strain of *M.tuberculosis* Erdman to compare the effect of disruption. The guinea pigs were euthanised three and eight weeks post-infection and the following parameters were analysed.

Various doses of these strains were administered subcutaneously into guinea pigs and then sacrificed three and six weeks after challenge. The viable count of tubercle bacilli isolated from the spleen and lung, the histopathological changes in the liver and the lung from these animals were determined. Experiments are in progress.

Protective efficacy of DNA vaccines in guinea pig tuberculosis

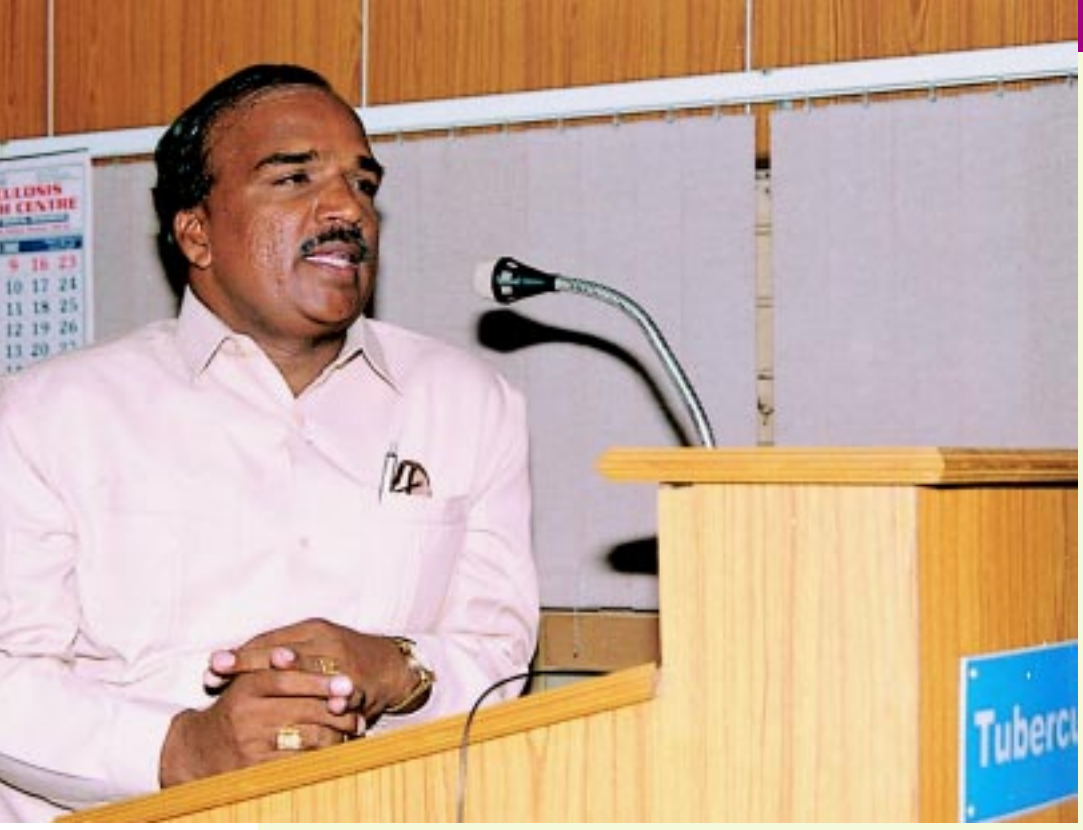
The DNA vaccine approach developed recently represents a novel strategy for the development of candidate vaccines against TB, especially since it is known to predominantly induce the Th1 arm of the immune system. Three DNA vaccine constructs expressing esat-6, µ-crystallin and SOD (superoxide dismutase) antigens of *M.tuberculosis* were injected into guinea pigs intramuscularly. This was followed by two booster injections of the same DNA constructs at three week intervals. Eight weeks after the last booster, all the animals were challenged with *M.tuberculosis* H_{37}Rv subcutaneously. The animals were euthanised at four and eight weeks post- challenge and the viable count of tubercle bacilli isolated from the spleen and lung and histopathological changes in the liver and the lung of these animals were determined. Experiments are in progress.

Staff list

V.D. Ramanathan, M.B.B.S., Ph.D.
Jaya Gopinath, B.Sc.,

Contact person

Cutaneous tuberculosis
Histopathology of tuberculosis
Immunohistochemical staining
Dr. V.D. Ramanathan
trcicmr@md3.vsnl.net.in(pathology)



Statistics

Neural networks and statistical modelling

Neural networks that appeared in the early 1980s established a new and popular branch of applied statistical modelling. Neural network models tend to be far more ambitious than traditional statistical models and more successful in large-scale problems. A simple neural network has three layers namely input layer, hidden layer and output layer. The input nodes–X_1, X_2, X_3, X_n– receive initial data values from each case. W_{ij} is the weight of connection between the i^{th}node in the input layer to the j^{th} node in the hidden layer. The inputs are transmitted to the next layer, i.e., the hidden layer along with its weight and bias term. Weight is the strength of connection and bias is a constant. A linear function of the inputs is given by $\sum W_{ij} X_i$. The linear sum is transformed to the hidden layer by applying an activation function, which is usually sigmoid or logistic. The value of the hidden unit, H_j , j = 1…..m is given by

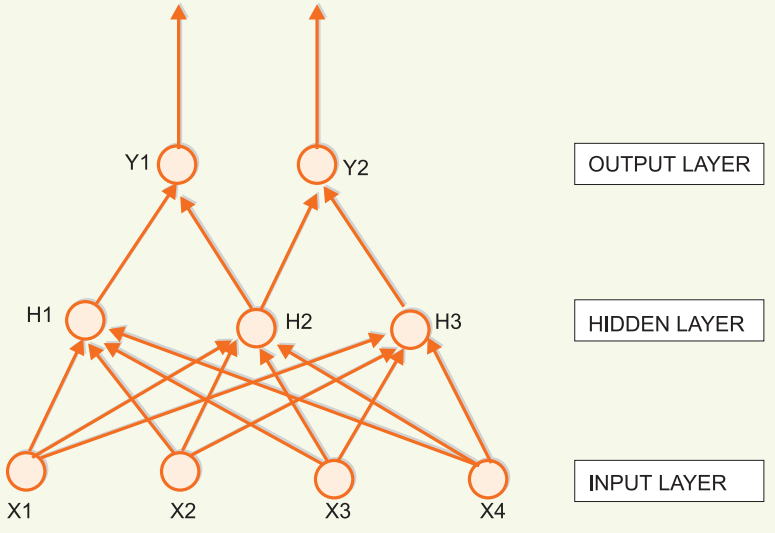
$$H_j = F_j\left(W_{0j} + \sum_{i-1}^{n} W_{ü} X_i\right) \text{----------} > (1)$$

where, f_j is the activation function of hidden unit and W_{oj} is the bias term connected to the j^{th} neuron. The value of the output unit, Y_k, k= 1……p is obtained by using another activation function f_k to the weighted sum of values of the hidden unit plus bias term of the hidden layer. Hence, the value of output layer is given by

$$Y_k = F_k\left(W_{0k} + \sum_{j-1}^{m} W_{j-k} H_j\right) \text{----------} > (2)$$

where W_{ok} is the weight of the bias term in hidden layer connected to the output layer and $\sum W_{jk} H_j$ is the linear function of hidden layer. A diagrammatic representation of a simple network (4 input, 3 hidden and 2 output units) is shown in Figure 18.

Fig. 18 Diagrammatic representation of feed forward neural network with two output units



The weights of the neural network are adjusted by a continuous process to solve the specific problem. Broadly, two types of learning are associated with neural networks, namely supervised and unsupervised learning. Supervised learning occurs when the target value is associated with each input in training data set. The actual output of the network is compared with the target value and the difference is used to correct or alter the weights. The most popular algorithm is the Back Propagation Algorithm, which uses supervised learning. The data set is split into test set and training set. Training set is used to train the network or to get the optimum weights and later is tested using the test set. Training starts with initial small values as weights and biases. The network is activated using equations 1 and 2 and the output is obtained. The output is compared with the desired/target values. The difference between the actual and desired output values is measured and the initial weights are changed by back propagating the error. The process is continued till the difference reaches the global minimal.

Regression methods have become an integral component of any data analysis concerned with describing the relationship between a response variable and one or more explanatory variables. Neural networks are used simultaneously with regression models to compare the efficacy and compatibility of these models. Neural networks can be viewed as a non-parametric regression method. One of the most popular regression models namely, the logistic regression for dichotomous outcome is compared here with neural networks.

Infant mortality data from a longitudinal study consisting of 2677 newborns for the period 1989-94 (Dr. J.Richard, CMC, Vellore) with an infant mortality of 160 (6 per cent) are used to compare the two models. Six variables namely, sex and birth weight of the infant (<2500g vs >=2500g); gestational age (<37w, 37-41w, >41w), education (illiterate, primary, others), occupation (housewife, others) and age (<20, 20-30, >30) of the mother are considered for model fitting, which is considered to influence the survival status of the infant. Of the 2677 newborns, 49 per cent were females, 80 per cent were above 2.5 kg weight, 15 per cent and 63 per cent had gestational ages <37 weeks and 37-40 weeks respectively. While 80 per cent of the months were housewives, one-third were illiterates. All the variables are duration independent. The comparison of two models are based on sensitivity, specificity and overall predictive ability of the model.

Table 18 presents the results of logistic regression adjusted for covariates. Only maternal education and birth weight are significant. The model

Table 18 Logistic regression for infant mortality – adjusted for covariates

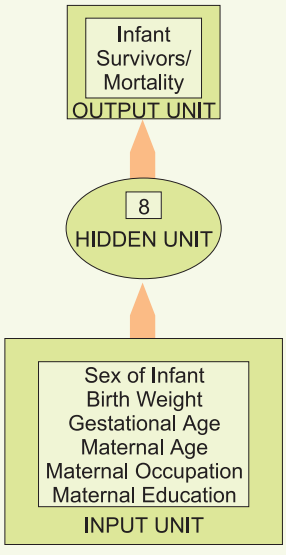
Variables	Coefficient	SE	O.R	95% C.I.
Sex				
Male (ref)	-	-	1.000	-
Female	1.2905	0.222	1.337	0.865-2.067
Gestational Age (weeks)				
<37	0.9417	0.275	1.407	0.821-2.413
37-41 (ref)	-	-	1.000	-
<41	-1.2533	0.304	0.776	0.428-1.409
Maternal Age (Yrs)				
<20	-0.9377	0.466	0.584	0.235-1.455
20-30 (ref)	-	-	1.000	-
<30	1.5673	0.294	1.764	0.991-3.135
Maternal Education (Yrs)				
0	2.0441	0.305	2.840**	1.563-5.160
1-5	2.9561	0.312	3.179**	1.732-5.863
6 & above (ref)	-	-	1.000	-
Maternal Occupation				
House Wife	-1.1625	0.272	0.850	0.479-1.454
Others (ref)	-	-	1.000	-
Birth Weight (g)				
<2500	0.9889	0.274	1.992*	1.164-3.408
>=2500 (ref)	-	-	1.000	-

* -P<0.05, ** -P<0.001

correctly classified 92.5 per cent of cases, using a cut-off point of 0.5. Sensitivity and specificity of the models were 37.9 per cent and 98.1 percent, respectively. The model explained 44% of variance (R^2 = 0.44).

Artificial neural networks (ANN) were constructed using back propagation algorithm and the schematic representation is given in Figure 19.

Fig. 19 Schematic representation of neural network in predicting infant mortality



The model correctly classified 96 per cent of the cases
Sensitivity = 70 per cent
Specificity = 94 per cent

The model explained 74 per cent of the variance.

Seventy-five per cent of cases were used as the training set and the remaining 25 per cent as the test set. A linear {-1,1} activation function was used for all inputs. A logistic function (0,1) was used as activation for the outputs, infant

survival / mortality. Eight hidden units were considered totally and were activated using a Gaussian function. About 520 learning epochs were performed. After training and testing, the important variables in the model that were associated significantly with the outcome were birth weight, maternal education and gestational age. ANN model correctly predicted 96 per cent of the cases. Sensitivity of the model was 70 per cent, which indicates moderate to high ability to link predictor with infant mortality. The specificity of the model was 94 per cent, which indicated high ability to link predictor with infant survival. The estimated explained variance was 74 per cent (R^2=0.74).

Various alternatives are being tested in the algorithm, including the number of hidden units and choice activation function to understand how ANN behaves for the same set of data under various conditions. The other works currently being carried out include comparison ANN with other semi-parametric (Cox's PH, AFT etc.), frailty and generalised additive models.

Staff list

P. Venkatesan, M.Phil., M.P.S., Ph.D., P.G.C.D.M., D.S.Q.C.O.R.(ISI), S.D.S.(ISI).
Fathima Rahman, B.Sc., Stat. Dip.(ISI)
N. Raghuraman, B.Sc.
Victor Mohan, B.Sc.
K. Thyagarajan, B.Sc.
M. Duraipandian, B.Sc.
B. Vaidyanathan, B.Sc.
K.G. Fredricks, B.Sc.
V. Sundaram, B.Sc.
V. Chandrasekaran, Ph.D.
A.S. Kripasankar, B.Sc.
R. Segaran, M.Sc.
K. Subramaniam, M.Sc.
M. Subhash Chandra Bose, M.Sc.
D. Suryanarayanan, M.Sc.
L. Sekar, M.Sc.
Shripad Bhat, M.Sc.
D. Vijaya Bhaskara Rao, M.A., M.Sc., M.Phil., Ph.D.
K. Chandrasekaran, M.Sc.
Naik Ashok Chandu, M.Sc.
C. Ponnuraja, M.Sc.
N. Arunkumar, M.Sc.

Contact persons

Statistical Methodologies
Survival Analysis
Dr. P. Venkatesan
trcicmr@md3.vsnl.net.in(stat)

Randomised Clinical Trials
Mrs. Fathima Rahman
trcicmr@md3.vsnl.net.in(mdp6fr)

Library & Information Division

The library and information division of the centre continues to cater to the felt need of the researchers of the centre and neighbouring institutions. The library started its online / electronic journal subscription during 2001. The centre subscribes to Online Journals Database, Health & Wellness Resource Centre, Gale Group, USA, that has a collection of more than 500 health / medical journals and hundreds of pamphlets, the Gale Encyclopedia of Medicine, Childhood, Adolescence, etc. The library has procured MEDLINE on CD-ROM since 1966. SDI service is being done through MEDLINE, NUCSSI, UCCDH and through internet. The library continues to act as a central node for electronic mail co-ordination agency for the staff of the centre. The centre has institutional membership facility with the British Council Library. We share our facilities and resources with the National Institute of Epidemiology. There is a plan to procure Snap Server to offer the electronic database search facility through LAN. Library automation process has been started and work is under progress.

Additions to the library during 2001:

Books	98
Journals (Electronic + Print)	566 (561+5)
CD-ROM	28

Staff and contact person

R. Rathinasabapathi, M.A., M.L.I.S.
trcicmr@md3.vsnl.net.in(library)

Administration & Support Services

Staff list

M. Subramaniam, B.Com.
C.J. Arunachalam
M.R. Srinivasan
Padma Balasubramanian
K. Jayarajan
Ranjith Sankar Sen
T.M. Kasinathan, B.Com., PG Dip. PM & IR
S.N. Sankaralingam, B.E.
E. Money
D. Ramani Bai
S. Vasantha
R. Ramamirtham
Samuel Swamidoss, B.A.
V. Lakshmanan
Santhi Velu, B.A.
D. Aruldoss, B.A.
P.N. Kalavathy Chari
M. Mani, B.A.
K. Kuppuswamy, B.A.
Jothi Segaran
Santha Sriraghavan
K. Saroja
P. Karthikeyan, B.Com.
K.S. Anusuya
Kanchana Udayakumar
V. Adikesavan, B.A.
P.K. Srinivasan, B.Sc.
B. Ramdoss, B.A.
N.C. Sridharan, B.Com.
M. Vijayalakshmi
A. Abdul Rahman, B.Sc.
K. Sampath Kumar, B.Sc.
D. Devaki, B.A.
K. Karunakaran, B.A.
Y. Samwilson, B.A.
B. Doraiswamy, B.A.
V.K. Venkatesan
T.S. Mahadevan
S. Rangamma
V. Lalithamma

Contact persons

Mr. M. Subramaniam

Sr. Administrative officer
trcicmr@md3.vsnl.net.in(admin1)

Mr. V. Adikesavan

Administrative officer (Epid)
trcicmr@md3.vsnl.net.in(admin2)

List of Publications

Publications in	(i)	International journals	: 15
	(ii)	National journals	: 14
Accepted for publication in	(i)	International journals	: 9
	(ii)	National journals	: 3

International

1. Sujatha Narayanan, Vijayalakshmi Parandaman, Narayanan, P.R., Venkatesan, P., Girish, C., Mahadevan, S., Sarala Rajajee.
   Evaluation of PCR using TRC_4 and IS6110 primers in detection of tuberculous meningitis.
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2. Cheruvu Mani, Selvakumar, N., Sujatha Narayanan, Narayanan, P.R. Mutations in the *rpoB* gene of multi drug-resistant *Mycobacterium tuberculosis* isolates from India.
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3. Immanuel, C., Rajeswari, R., Rahman, F., Paul Kumaran, P., Chandrasekaran, V., Swamy, R. Serial evaluation of serum neopterin in HIV seronegative patients treated for tuberculosis.
   International Journal of Tuberculosis and Lung Disease, 2001, **5**, 185-190.

4. Chandra Immanuel, Hemanth Kumar, A.K. Simple and rapid high-performance liquid chromatography method for the determination of ofloxacin concentrations in plasma and urine.
   Journal of Chromatography, 2001, **760**, *91-95*.

5. Uma Devi, K.R., Ramalingam, B., Brennan, P.J., Narayanan, P.R., Alamelu Raja. Specific and early detection of IgG, IgA and IgM antibodies to *M. tuberculosis* 38 kDa antigen in pulmonary tuberculosis.
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International Journal of Tuberculosis and Lung Disease, 2001, **5**, 40-45.

10. Radhakrishna, S., Thomas R Frieden, Subramani, R., Paul Kumaran, P. Trends in the prevalence and incidence of tuberculosis in South India.
International Journal of Tuberculosis and Lung Disease, 2001, **5**, 142-157.

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Protein Expression and Purification, 2002, **24**, 188-195.

12. Alamelu Raja, Uma Devi, K.R., Ramalingam, B., Patrick J Brennan. Immunoglobulin G, A and M response in serum and circulating immune complexes against 16 kDa antigen of *M. tuberculosis*.
Clinical and Diagnostic Laboratory Immunology, 2002, **9**, 308-12.

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14. Daisy Vanitha, J., Chandra Immanuel, Bogumilla Szponar, Lennart Larsson, Paramasivan, C.N. Identification of a group of non tuberculous mycobacteria isolated from the South Indian BCG trial area by HPLC.
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13. Swaminathan S. Clinical presentation and treatment of HIV/TB. *Indian Journal of Tuberculosis*, 2002, **49**, 11-16.

14. Tuberculosis Research Centre Shortening short course chemotherapy : a randomized clinical trial for the treatment of smear positive pulmonary tuberculosis with regimens using ofloxacin in the intensive phase. *Indian Journal of Tuberculosis*, 2002, 49, 27-38.

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1. Uma Devi, K.R., Ramalingam, B., Alamelu Raja. Qualitative and quantitative analysis of antibody response in childhood tuberculosis against antigens of *Mycobacterium tuberculosis*. *Indian Journal of Medical Microbiology*.

2. Saraswathy, S.D., Prema Gurumurthy, Devaraj, H., Jaganathan, K., Shyamala Devi C.S. Effect of Liv.100 on antioxidant status in patients administered with different anti-TB drug regimens. *Biomedicine.*

3. Soumya Swaminathan, Sangeetha, M., ArunKumar, N., *et al.* Clinical, radiological and immunological profile of pulmonary tuberculosis in HIV-infected persons. *Indian Journal of Tuberculosis.*

Participation in Conferences / Workshops / Seminars

The centre has provided opportunities to its staff members (research and technical) for their professional development through participation in national and international level workshops, conferences, seminars and training programmes such as:

International symposium on mycobacterial diseases: Pathogenesis, Protection and Control - Kolkata - Jan. 2001

Nuffield council consensus meeting on Biomedical ethics - Chennai - Jan. 2001

SAARC - WHO meeting of potential consultants in TB control and review draft guidelines for preparation of 5-year TB control plans - Kathmandu - Jan. 2001

Workshop on PCR based-methods in disease diagnosis - Chennai - Feb. 2001

5th International Congress of Diabetes Society - Chennai - Feb. 2001

Global Alliance for TB Drug Development Conference- R & D Coalition for TB Drug Development in Asia - Penang - May 2001

WHO meeting on Protocol development ; Multi centric study of delay in diagnosis - Geneva - May 2001

Annual Conference on the Role of families in preventing and adapting to HIV/AIDS - Los Angeles - July 2001

Conference of Indian Association of Medical Microbiologists (Tamil Nadu and Pondicherry) - Pondicherry - July 2001

Workshop on Information Technology in Bio-Medical Research - Chennai - Aug. 2001

WHO-ICMR workshop in Clinical Pharmacology - Mumbai - Sept. 2001

Indo-German workshop on Genetic Basis of host-pathogen interactions - Chandigarh - Sept. 2001

Management development programme on digital libraries - Kozhikode - Sept. 2001

International Conference on stigma and global health: Developing a research agenda- Maryland - Sept. 2001

56th National Conference on Tuberculosis & Chest Diseases - Chennai- Oct. 2001

National Study Conference on Human rights and refugees - Chennai - Oct. 2001

28th Annual Conference of the Indian Immunology Society & Symposium - New Delhi - Oct. 2001

13th National Pediatric Pulmonology meet - Thiruvananthapuram - Oct. 2001

The 32nd IUATLD World Conference on lung health - Paris - Nov. 2001

International Conference on Medical informatics - Hyderabad - Nov. 2001

16th Asia Pacific Congress on Diseases of the chest (APCDC) - Mumbai - Nov.2001

19th Annual Conference of Indian Society for Medical Statistics & 6th Conference of the International Biometric Society (IR) - Lucknow - Dec. 2001

Ramanujan Memory National Seminar on Mathematical methods and applications - Chennai - Dec.2001

International symposium on Current developments in drug research for tuberculosis. Astra Zeneca Research Foundation - Bangalore - Jan. 2002

9th Conference on Retroviruses and opportunistic infections - Seattle - Feb.2002

WHO-UNAIDS sponsored Workshop on Application of a novel gag/HMA technique for determination of HIV-1 genetic subtypes and inter-subtype recombinants in Asia- Bangkok - Feb. 2002

Indo-French symposium on Tuberculosis and AIDS - Chennai - Mar. 2002

Tuberculosis Research Centre
Mayor V.R. Ramanathan Road
Chetput, Chennai - 600031
India
Telephone : +91 44 8265425/27/35/57
Fax : +91 44 8262137/8228894
E-mail : trcicmr@md3.vsnl.net.in
icmrtrc@vsnl.com
Website: www.trc-chennai.org