INDIAN COUNCIL OF MEDICAL RESEARCH

TUBERCULOSIS RESEARCH CENTRE

Chennai

Report on Research Activities 1999-2000

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Contents

Message from Director General

आचार्य एन.के. गांगुली
महानिदेशक
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MD, FRC Path (London), FAMS, FNA, FASc, FNASc
Director General

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Indian Council of Medical Research
Ansari Nagar, Post Box 4911, New Delhi - 110 029

MESSAGE

The **Tuberculosis Research Centre (TRC)** is one of the premier institutes of the Indian Council of Medical Research (ICMR). The Centre has over the last four decades successfully fulfilled its mandate to develop new chemotherapeutic tools that have played an important role in the development of control strategies for tuberculosis not only in India but in other countries as well. The TRC has a fine reputation for the development of research methodologies for the conduct of controlled clinical trials and large-scale epidemiological studies.

The Council desires to develop TRC into an international centre of excellence in medical research. In order to achieve this TRC will need to develop and acquire newer capabilities in the field of modern biology relevant to tuberculosis research. I am sure that the staff and the Director of the Institute will rise up to the challenge of achieving this goal.

I am pleased to learn that the Tuberculosis Research Centre is packaging and disseminating its research activities in a new-look format. Research that is not disseminated and utilized has no value and I am glad that the Centre has carefully packaged its information in an informative and attractive fashion.

I have pleasure in placing this copy of the **Annual Report** before you.

(N.K.Ganguly)

Preface

Have we made an impact?

Tuberculosis is curable, but in India it continues to kill one person every minute. With a predilection for the breadwinners of families, this disease pushes many families deeper into poverty, ravaging much more than its victims' individual health. Tuberculosis is truly an economic as well as a public health burden.

Tuberculosis is also a controllable disease — a fact that has been demonstrated in many parts of the world with existing tools. Yet it strikes 2 million people each year in our country exacting a toll higher than many tropical diseases put together.

The transmission, diagnosis and treatment of tuberculosis are well characterized. Powerful anti-tuberculosis drugs have been available for half a century. Elements of effective strategies for control have been known for several decades. Nonetheless, tuberculosis control is still a dream for many countries, including India.

Ultimately, institutions and organizations involved in research and control of tuberculosis are accountable for such lapses. The Tuberculosis Research Centre (TRC), an institution created specifically for research purposes more than four decades ago, is no exception. Now it is time to ask ourselves: **have we made an impact?**

In reality, our impact is best measured at the level of the patient care provider, the laboratory worker, the public health specialist and other "end users" who depend on our research for effective tuberculosis control. A yawning chasm exists between the requirements of programme managers and the needs of clinic- and laboratory-based health care providers. The public health specialist needs simple diagnostics, fail-safe treatment and robust monitoring and evaluation — in other words, tools that can be used for large populations. Needless to say, these tools must be simple and inexpensive. On the other hand, individual patient care providers crave higher-tech, state-of-the art instruments for individualized diagnosis and treatment, regardless of price.

As a research organization, we also need to satisfy the needs of our parent body, funding agencies, and donors. These entities' requirements, as well as their benchmarks of success, often diverge. Research at the "cutting edge of science" (e.g., genomics, proteomics, and vaccinology) is publishable in prominent international peer-reviewed journals and convertible to international patents but it may not benefit the programme in the long- or short-term.

I believe that the TRC has effectively served the needs of public health not only in this country but also globally; we have made an impact. Over the past four decades, the TRC has made important contributions to the treatment of tuberculosis. Most of the tools used in tuberculosis evolved in India, primarily at the TRC. Stefan Grzybowski, a renowned tuberculosis epidemiologist, summed up the TRC's role thusly: "India has taught the world that hospitalization is not essential for the treatment of tuberculosis, the principles of chemotherapy of tuberculosis, supervised intermittent chemotherapy and the supreme importance of bacteriological diagnosis." DOTS, the globally recommended strategy

for tuberculosis control now used in more than 120 countries, is to a large extent a product of the TRC. In the field of preventive research, the Chingleput BCG trial has no parallel. The results of this extraordinarily complex trial helped formulate the vaccine policy of this country and many others worldwide. Unfortunately, these lessons do not always translate into better tuberculosis control in our country; as noted by Dr. Halfdan Mahler, a former Director General of the World Health Organization, "All countries benefit from the fruits of Indian TB research — all countries except India."

It is not enough to rest on past successes. Over the past four years, the TRC has made significant contributions in the area of capacity building and advocacy for the control of tuberculosis in this country. Our entry into the area of training, quality assurance, and operational research has made a tremendous difference to the programme managers of this country. A recently published study from the TRC quantified, for the first time, the socioeconomic burden of tuberculosis in this country. The paper has become a landmark, being publicly quoted by the highest political authorities in the country as well as by international agencies. Our efforts to bridge the gap between public and private sector partners in tuberculosis control have also been appreciated internationally. The TRC's website has been rated the best among tuberculosis sites in the region for its content and design. Undoubtedly we have made an impact in operational research and the dissemination of research findings.

We have also focussed our efforts on some of the most essential needs for tuberculosis control: diagnosis, treatment and monitoring. For diagnosis, research at the TRC in the past year has documented that simple and readily available chemicals may greatly increase the sensitivity and acceptability of sputum microscopy. For treatment, a controlled trial has shown that it is possible to reduce the length of anti-tuberculosis treatment to 4 months — a finding that is likely to have a global impact in the near future. And for monitoring, the TRC has emerged as the nodal institute for national drug resistance surveillance, monitoring the baseline and ongoing impact of treatment programs on the critical indicator of drug resistance. In addition, the Model DOTS Project will establish for the first time in a developing country the potential epidemiologic impact of effective tuberculosis control on the incidence and prevalence of tuberculosis infection and disease.

In the area of basic science, however, our impact lags somewhat. In part, the TRC has struggled with difficulties specific to tuberculosis and encountered by researchers throughout the world. *Mycobacterium tuberculosis* is a complex organism with an array of seemingly inscrutable survival techniques. For this reason, there have been no significant breakthroughs from any lab in the world in the areas of tuberculosis diagnosis and vaccination. The most widely used diagnostic tool, smear microscopy, is more than a century old, and the only vaccine available is three-quarters of a century old. Moreover, during the second half of the past century, the effectiveness and ready availability of intervention strategies for tuberculosis control have shrunk the impetus for basic science and product development. In fact, so complacent has been the attitude of all concerned with tuberculosis management and control that the newest drug for tuberculosis treatment was developed almost forty years ago.

However, the spread of HIV/AIDS and multidrug-resistant tuberculosis have rekindled the interest of researchers and programme personnel. The recent unravelling of the genomic structure of *M. tuberculosis* presents a fantastic opportunity to develop new diagnostics, drugs and vaccines.

Realistically speaking, however, such opportunities can be exploited only by technologically advanced laboratories. And technology does not come cheap. It calls for funds, equipment, technical skills, energy and enthusiasm. In most institutes, these assets are in short supply.

How then can the TRC make an impact in basic sciences? We have begun by restructuring. We have created a division of HIV/TB that will address both basic and applied problems in this area. We have also expanded our laboratory interests to include molecular biology and molecular epidemiology. Over the past three years, we have convened a series of international and Indian workshops (India-EU, India-US-Japan, India-France) to identify frontier areas for collaborative efforts. But we need people with new skills to join our team if we are going to effect substantial changes in the next decade.

Overall, I do believe that the TRC has made a significant impact. All medical research should have as its ultimate goal disease control, and it cannot be justified unless the new knowledge and technology are disseminated and utilized. In this sense, the TRC has excelled; its effects have been greatest in the fields where it matters most — public health, epidemiology and research methodology.

But we cannot stop with our past or current achievements, no matter how impressive. Much work remains ahead of us in tuberculosis control. I am convinced that this can be achieved only by forging partnerships locally, nationally and globally – an approach the TRC has consistently advocated.

I seek your advice and support in helping me to restructure, to build a new TRC that can continue its excellent work, delve into newer areas and bid competitively for funds. This annual report is proof of our intentions. Given your support, I am sure that we will continue to provide new knowledge and tools that are worthy of our status as a flagship institute of the Indian Council of Medical Research.

P.R. Narayanan

Director
Tuberculosis Research Centre

Vision Statement

To develop the Tuberculosis Research Centre into

- **a centre of excellence for research in tuberculosis**
- **an opinion leader on tuberculosis control policies**
- **a core facility for training for tuberculosis research and control**
- **a nodal agency for advocacy for tuberculosis control in India**

Mission Statement

- **TRC is mandated by the Indian Council of Medical Research to provide scientific understanding and technologies needed to support the fight against tuberculosis.**

- **TRC supports and promotes "DOTS" in the Revised National Tuberculosis Control Programme of the Government of India by providing better tools and refining existing tools for diagnosis, treatment and monitoring of tuberculosis through controlled clinical trials and scientific research.**

- **TRC strives for integrity, quality and relevance of its research by constantly improving its scientific programs through external peer review and competitive funding.**

- **TRC utilizes tools from clinical, biological and social sciences to understand the parasite, patient and the programme associated with tuberculosis.**

- **TRC recognizes its obligation to patients with tuberculosis and promotes and practices the highest standard of patient care in the course of its research activities.**

- **TRC develops statistically reliable protocols for the evaluation of intervention strategies for the control of tuberculosis.**

- **TRC endeavours to provide excellent training programs to researchers and programme personnel in both basic and clinical sciences.**

- **TRC aims to bridge the gap between the public establishment and private enterprise, to promote advocacy required for the control of tuberculosis.**

- **TRC is committed to the dissemination of knowledge and leads in the development and use of the technologies needed to synthesize, analyze and disseminate information about tuberculosis.**

- **TRC enters into partnerships with local, national and international agencies to develop sustainable strategies for the control of tuberculosis.**

- **TRC respects the ethical principles associated with research and strives to safeguard its intellectual property rights and protect the rights of others.**

Tuberculosis Research Centre down the years...

The Tuberculosis Research Centre (TRC), formerly known as Tuberculosis Chemotherapy Centre is a permanent research institute of the Indian Council of Medical Research (ICMR), an autonomous organization under the Ministry of Health and Family Welfare, Government of India. It was established in 1955 jointly by the ICMR, World health Organization (WHO), the Government of Tamil Nadu (then Madras) and United States Public Health Service (USPHS) to determine the feasibility, efficacy and safety of domiciliary chemotherapy for sputum positive pulmonary tuberculosis patients. Having established this, the Centre then conducted randomized controlled clinical trials to evaluate principles of chemotherapy for tuberculosis, both pulmonary and extra pulmonary forms. These studies were undertaken in collaboration with the governmental and non-governmental agencies. Over the years TRC has evolved regimens that have reduced adverse reactions, allowed monitoring of drug intake and prevented concealed drug irregularity. Many of these studies of the Centre have formed the basis of the current globally acclaimed strategy of DOTS.

In 1983, the Centre expanded its activities and helped to introduce SCC in 18 districts, spread over 10 States all over India and one Union Territory, under the then existing District Tuberculosis Programme conditions. Operational research studies identified the potential of DAIS (traditional birth attendants), NSS student volunteers and literate youth volunteers to improve case finding and case holding of the programme.

TRC has also been associated with the conduct of the renowned Chingleput BCG trial that is recognized as one of the largest and best conducted clinical trial in the world. Other major studies done in this area include the Leprosy Prevention Trial and drug resistance surveillance.

Well-equipped laboratories ably support the clinical activities. The Centre is recognized for its contributions to mycobacteriology and its current research efforts in this field involve characterization of atypical mycobacteria, studies on development of drug resistance and dormancy. The Centre is recognised as an international referral laboratory and is also a designated WHO collaborating centre for tuberculosis research and control. The Centre has developed novel methods for assaying anti-TB drugs by estimating drug levels in body fluids other than blood and is involved in bioavailability studies. It has also identified novel promoters of mycobacteria that show potential for drug and vaccine development. The Centre has defined several HLA and non-HLA polymorphisms associated with pulmonary and extra-pulmonary tuberculosis. It is engaged in developing immunodiagnostics to facilitate early diagnosis and is a major centre for evaluation of diagnostic tools for tuberculosis. More recently it has initiated molecular epidemiological studies, which will not only supplement conventional epidemiological information but also provide insights into transmission dynamics of the disease.

Although the mandate of the centre initially was primarily research in the field of tuberculosis, the Centre over the years expanded its activities to encompass training also. Since 1998 TRC is providing extensive training to various categories of personnel of the Revised National Tuberculosis Control Programme (RNTCP). Thus, the governments of India, Tamil Nadu and various other agencies have exploited the vast experience of the scientists of the TRC for these training programmes.

At the turn of the millennium the Centre is justifiably recognized internationally as the leader in the field of tuberculosis research and control by virtue of its contributions to research, training and patient care.

TRC's contribution to TB control programme

Subject	Problem	Research outcome	Impact on control/ Research utilzation
Home-Sanatorium study	Inadequate beds for TB patients Risk of TB in contacts	TB patients can be safely treated at home without risk to contacts	Released beds in sanatoria and reduced capital costs Foundation for DOTS
Single dose INH	Supervision of divided doses difficult	Single doses of INH superior to divided doses	Made possible supervision of all doses Foundation for DOTS
Toxicity of INH	Peripheral neuropathy in TB patients on INH treatment	Pyridoxine deficiency responsible for neuropathy Role of acetylator phenotypes identified	Increased compliance due to addition of pyridoxine to regimens
Supervised intermitent chemotherapy	Concealed irregularity Daily supervision difficult	Twice weekly treatment as good as daily treatment First intermittent chemotherapy study in the world	Reduced drug toxicity and costs Foundation for DOTS
Once weekly chemotherapy	Twice weekly treatment difficult Toxicity due to streptomycin	Once weekly treatment not effective 0.75gm streptomycin is effective	Decreased toxicity and cost of treatment due to streptomycin
hiacetazone stud	Combination of para-amino salicylic acid and INH bulky and costly	Thiacetazone - INH combination is as good as para-aminosalicylic acid and INH	Reduced bulk and cost of drugs
Short course chemotherapy (SCC)	Treatment duration too long Non-compliance	Treatment reduced to 5-7 months Reduction of treatment below 5 months not acceptable Rifampicin introduced in India	Decreased organisational costs Enhanced compliance
Regimens for management of relapse	Choice of drugs for management of relapses	Relapse cases have drug susceptible organisms Retreatment is not a problem	Sensitivity testing not required in field Cost of relapse mangement reduced
Extra-pulmonary TB	No guidelines on value of SCC	6-9 months SCC is effective Stage of diagnosis critical in the management of tuberculous meningitis	Defined guidelines for SCC in extra-pulmonary TB Decreased duration, toxicity and cost of treatment Role of surgery defined for spinal TB
Community volunteer mobilisation for TB control	Poor access to diagnostic and treatment facilities	Community volunteers can provide reliable and accurate assistance for TB control	Potential service providers identified Minimal training required for enlisting their support
BCG trial	Efficacy of BCG unknown	Largest field trial to date BCG does not protect against adult forms of TB	Allowed introduction of BCG vaccination into expanded programme of immunization Developed TB field epidemiology methods
SCC in National Programme	Efficacy of SCC under field conditions unknown	Less than half the patients are cured	Identified need for DOTS

Scientific Advisory Committee

Ex-officio Chairman

Prof.N.K.Ganguly	Indian Council of Medical Research, New Delhi.

Chairman

Dr.C.M.Gupta	Central Drug Research Institute (CSIR), Lucknow.

Members

Dr.D.A.Gadkhari	National Institute of Virology (ICMR), Pune.
Dr.K.N.George	Madras School of Social Work, Chennai.
Prof. Indira Nath	All India Institute of Medical Sciences, New Delhi.
Dr.K.Jaganath	Institute of Thoracic Medicine, Chennai.
Dr.P.Jagota	National Tuberculosis Institute, Bangalore.
Dr.C.S.Jayachandran	Directorate of Medical Education, Government of Tamil Nadu, Chennai.
Dr.(Capt) M.Kamatchi	Directorate of Medical Sciences, Government of Tamil Nadu, Chennai.
Dr.Lalit Kant	Indian Council of Medical Research, New Delhi.
Dr.G.R.Khatri	Directorate of Health Services, (Central TB Division), New Delhi.
Dr.J.N.Pande	Department of Medicine, All India Institute of Medical Sciences,New Delhi.
Dr.S.C.Sehgal	Regional Medical Research Centre (ICMR), Port Blair, Andamans.
Dr.U.Sengupta	JALMA, Institute of Leprosy (ICMR), Agra.
Prof.K.V.Thiruvengadam	41, G.N.Chetty Road, T.Nagar, Chennai.
Dr.Anil K.Tyagi	University of Delhi (South Campus), New Delhi.
Dr.M.W.Uplekar	Foundation for Research in Community Health, Mumbai.
Dr.V.Vijayasekaran	35, Thirumurthy Street, T.Nagar, Chennai.

Member-Secretary

Dr.P.R.Narayanan	Tuberculosis Research Centre (ICMR), Chennai.

Ethical Committee

Chairman

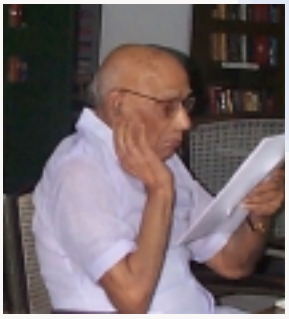
Justice N.Krishnaswamy Reddy	Justice (Retd.), Madras High Court, Chennai.

Members

Dr.K.N.George	Director, Madras School of Social Work, Chennai.
Prof.(Mrs).Lalitha Kameswaran	Vice Chancellor (Retd.), The Tamil Nadu Dr.MGR Medical University, Chennai.
Dr.H.Srinivasan	Honorary Editor, Indian Journal of Leprosy, Chennai.

All research proposals involving human subjects (or human tissue) are screened by the Ethics Committee of the Centre. The Committee examines the ethical and scientific acceptability of the projects. The Centre's projects especially in the field of epidemiology and clinical trials raise complex ethical issues which have been extensively discussed at the ethical committee meetings. The Committee utilizes the guidelines prescribed by the Indian Council of Medical Research to assess the ethical acceptability of project proposals.

During 1999-2000 the Ethics Committee reviewed 10 new proposals including studies to assess the epidemiological impact of DOTS, diagnostics algorithms and chemoprophylaxis of HIV/TB, helminth and mycobacterial co-infections.



"...Ethical issues are now real: don't take them for granted. Informed consent must be truly informed.....inform patients and the ethical committee of the progress of research projects..."

- Justice(Retd.) N.Krishnaswamy Reddy

"...patients cannot be treated as guinea pigs. The potential toxicity of drugs used as chemoprophylactic agents must be examined carefully.....the same standards must be used for developed and developing countries..."

- Prof.(Mrs.) Lalitha Kameswaran

"...epidemiological studies should take into consideration the long-term perspectives of the community's health.....the quality of care offered to patients in the research study should be higher than that offered under programme conditions..."

- Dr.H.Srinivasan

Institutional Committees

ETHICAL COMMITTEE (Internal)

Dr.Rema Mathew (Chairperson)
Dr.M.Kannapiran
Dr.Alamelu Raja
Dr.C.Kolappan
Mrs.Sudha Ganapathy

BIO-SAFETY COMMITTEE

Dr.C.N.Paramasivan (Chairperson)
Dr.V.D.Ramanathan
Dr.Sujatha Narayanan
Dr.Naseema
Dr.M.S.Jawahar
Mr.Abdul Ravoof

PURCHASE COMMITTEE

Dr.H.N.Madhavan (Chairperson)
Director of Research,
Vision Research Foundation, Chennai.

Dr.Radha Madhavan
Prof. of Immunology,
Madras Medical College, Chennai.

Dr.Soumya Swaminathan
Dr.Paulin Joseph
Dr.M.Kannapiran

BUILDING COMMITTEE

Dr.C.N.Paramasivan (Chairperson)
Dr.M.S.Jawahar
Dr.V.D.Ramanathan
Dr.Rema Mathew
Dr.P.Venkatesan
Mr.M.Subramanian
External Member (Not Below the Rank of Executive Engineer - PWD)

VIGILANCE COMMITTEE

Dr.C.N.Paramasivan (Chairperson)
Mrs.Chandra Immanuel
Dr.D.Baskaran
Mr.R.S.Sen (Member Secretary)

CONDEMNATION COMMITTEE

Dr.Prema Gurumurthy (Chairperson)
Mr.S.N.Sankaralingam
Mr.E.Money
Mr.C.J.Arunachalam (Member Secretary)

LIBRARY COMMITTEE

Dr.V.Kumaraswami (Chairperson)
Dr.Rema Mathew
Dr.Sujatha Narayanan
Dr.K.V.Kuppu Rao
Mr.R.Rathinasabapathy (Member Secretary)

ANTI-WOMEN HARASSMENT COMMITTEE

Dr.Usha Ramanathan (Chairperson)
Dr.P.Selvaraj
Mrs.Sara Mathew
Mr.V.Adikesavan

Distinguished Visitors

"... the great expertise available here should be utilised to explore possible future studies in HIV/AIDS..."

John L. Fahey
UCLA School of Medicine,
Los Angeles,California,
USA.

"... this institute should be a centre for worldwide research on TB both from basic as well as clinical point of view..."

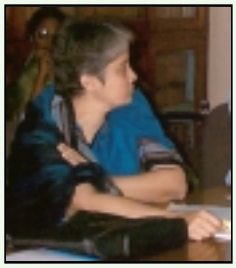
Masao Mitsuyama
Professor and Chair
Department of Microbiology,
Kyoto University School of Medicine,
Japan.

"...(TRC) place of current and historical importance and impressive range of research..."

Martien W. Borgdorff
Epidemiologist,
KNCV,
The Netherlands.

"...anticipating support and collaboration in TB research with TRC..."

Claude Griscelli
Director General of INSERM,
France.



"...impressed by the research activities going on in the centre..."

Shoba Koshy
Director, Ministry of Health and Family Welfare,
New Delhi, India.

"...an institute of international standards...
I think more doctors should visit and get benefited..."

Nural Anowar
Director, UMIS,
D.G.H.S. Monakhali,
Dhaka, Bangladesh.

"...the contributions made by the centre to the challenges of
TB control are highly commendable..."

Sue Emmott
Governance Advisor, DFID,
New Delhi, India.

"...staff here are very competent and have
expertise in the field of TB research..."

A.S.M. Kamal
Director,
Planning and Research,
Ministry of Health & Family Welfare,
Bangladesh.

Mrs.Kamala Malhotra
Dy.Leader of the House,
Chairman, Medical Relief &
Public Health Committee.

Mrs.Bimla Sharma
Dy.Chairman,
(Shahdara North Zone).

Mrs.Vijay Vahal
Municipal Member,
Delhi Nagar Nigam.

"...educating people about tuberculosis is very important and we are confident that Tuberculosis Research Centre will succeed in achieving this goal..."

R.S.Sisodia
State TB Officer,
Rajasthan.

"...wonderful; masterpiece educative presentation on various research issues..."

"…the disease survey carried out by TRC is of very high quality..."

A.Siri Baddana
Chest Physician,
Teaching Hospital,
Kandy, Sri Lanka.

UPGRADATION OF FACILITIES

Rainarain Conference Room

Robert Koch Auditorium

Fox & Mitchison Room

UPGRADATION OF FACILITIES

Sanjeevi Conference Room

Ziehl Neelsen Room

Guld Computing Room

ICMR/WHO National Workshop on Health Research Management September 14-16, 1999

A National Workshop on Health Research Management sponsored by the Indian Council of Medical Research and the World Health Organization and organised by Tuberculosis Research Centre was held at Chennai on September 14-16, 1999. The workshop brought together researchers from the major health research institutes, universities, NGOs, private sector, industry and programme managers. The main objectives of the workshop were to (a) review the status of health research management in India, (b) identify methods to streamline and improve health research management, (c) identify and harness newer technologies for better research management and (d) discuss methodologies for establishment of better research management practices. The workshop was inaugurated by Dr. K. Ananda Kannan, Vice-Chancellor, The Tamil Nadu Dr. M.G.R. Medical University, Chennai.

Workshop on Involvement of Private Practitioners in RNTCP November 12-13, 1999

A two-day workshop was organised by Tuberculosis Research Centre on November 12-13, 1999 in collaboration with the State Health Department, Govt. of Tamil Nadu, Ministry of Health & Family Welfare, Govt. of India & Lala Ram Sarup Institute of Tuberculosis and Allied Diseases, New Delhi, with financial assistance from the World Health Organisation. Shri Deepak Gupta, Joint Secretary, Ministry of Health and Family Welfare, who was instrumental in bringing about this workshop, emphasised the need for participation of Private sector in RNTCP.

Representatives from Tamil Nadu Voluntary Health Associations, Lions Club International, Madras District, Advocacy for Control of Tuberculosis and the Indian Medical Association spoke on the various activities of their groups and also their involvement in TB control.

5th International Conference on Emerging Infectious Diseases in the Pacific Rim January 7-9, 2000

This meeting was the fifth in a series of scientific meetings sponsored by the United States-Japan Cooperative Medical Science Program to explore and discuss the scientific approaches needed to address the problem of emerging and re-emerging infectious diseases. Over 150 leading scientists from USA, Japan, Pacific Rim countries and India participated. The Indian delegation was headed by Prof.N.K. Ganguly, Director General, Indian Council of Medical Research while Dr.Tadao Shimao was the chairperson of the Japan delegation and Dr. Carpenter was the leader of the US delegation.

The organizing committee for this meeting focussed discussion on three topics – tuberculosis, leprosy and HIV/AIDS. The conference recommended that research infrastructure in the region in the areas such as research training, specialised reagents, research infrastructure for basic, epidemiological and clinical research, information resources (eg.Internet access), research and communications networks and disease surveillance need to be strengthened.

INDO-US Conference on HIV/AIDS Prevention Research January 11-12, 2000

The Indian Council of Medical Research (ICMR) and the U.S. National Institutes of Health (NIH) organized the Indo-U.S. Conference on HIV/AIDS Prevention Research on January 11-12, 2000. Prof. N. K. Ganguly, Director-General, ICMR, Dr. Gerald T. Keusch, Director, Fogarty International Center and Dr. Bernard Alter, U.S. Consul General in Chennai highlighted the need for Indo-U.S. coordinated research efforts for HIV/AIDS prevention and control. Dr. Keusch noted the growing prominence of global health issues, mentioning that for the first time the United Nations Security Council had considered the importance of health related issues, in particular, HIV/AIDS. Prof. N. K. Ganguly placed special emphasis on the ongoing collaboration between Indian and U.S. scientists in this area.

Working groups addressed the primary research questions in four areas: behavioral prevention research, other biomedical prevention research, HIV vaccine research and prevention of vertical transmission of HIV.

Protocol development workshops for Private Sector and HIV November 1-2, 2000

A two day workshop was jointly sponsored by Tuberculosis Research Centre, ICMR and Central TB Division, DGHS. A total of 26 participants from different parts of India attended the 2-day workshop at TRC. The participants included representatives from Medical colleges, Non-governmental organizations, DTOs, TRC, NTI and Dr.Khatri DDG, DGHS.

The following protocols for multicentric studies were developed.

1. A common protocol for conducting operational research in involvement of private sector in RNTCP which can be applied in any area in India

2. Assessment of RNTCP regimens in the treatment of TB patients with HIV.

3. Chemoprophylaxis for tuberculosis among HIV infected patients.

INDO-FRENCH Workshop on Tuberculosis December 1-2, 2000

In pursuance of the Memorandum of Understanding (MOU) between the Indian Council of Medical Research and INSERM, signed on 1st February 1989, for strengthening mutual understanding and co-operation for research in biomedical sciences, a meeting of the Directors General of ICMR and INSERM, along with their respective teams was held at New Delhi on 28th January 2000. As a follow up of this meeting, a workshop on Tuberculosis was held on December 1 and 2, 2000 at the Tuberculosis Research Centre, Chennai.

Over 40 leading French and Indian scientists working in the field of tuberculosis participated in the meeting. The topics covered during the workshop included mycobacterial genomics, molecular epidemiology, molecular aspects of metabolism, molecular aspects of virulence and immunodiagnosis of tuberculosis.This was followed by discussions regarding partnerships and future action.

National Workshop on the Revised National Tuberculosis Control Programme for the Medical College Faculty
October 5-6, 2000

A successful national programme must have the enthusiastic support of a well-informed medical profession. Because of the novel aspects of the Revised National Tuberculosis Control Programme (RNTCP), medical education needs to be updated and medical teachers need to be sensitized to the new programme. To address these needs, a national workshop on the RNTCP for teachers in Medical Colleges was organized and hosted by the Tuberculosis Research Centre in Chennai on October 5-6, 2000. The workshop was sponsored by the Indian Council of Medical Research and the World Health Organisation. The main objectives of the workshop were to define the desired curriculum content and learning processes of undergraduate and post graduate medical students, so that as future medical practitioners, they can contribute effectively to the management of tuberculosis control programmes.

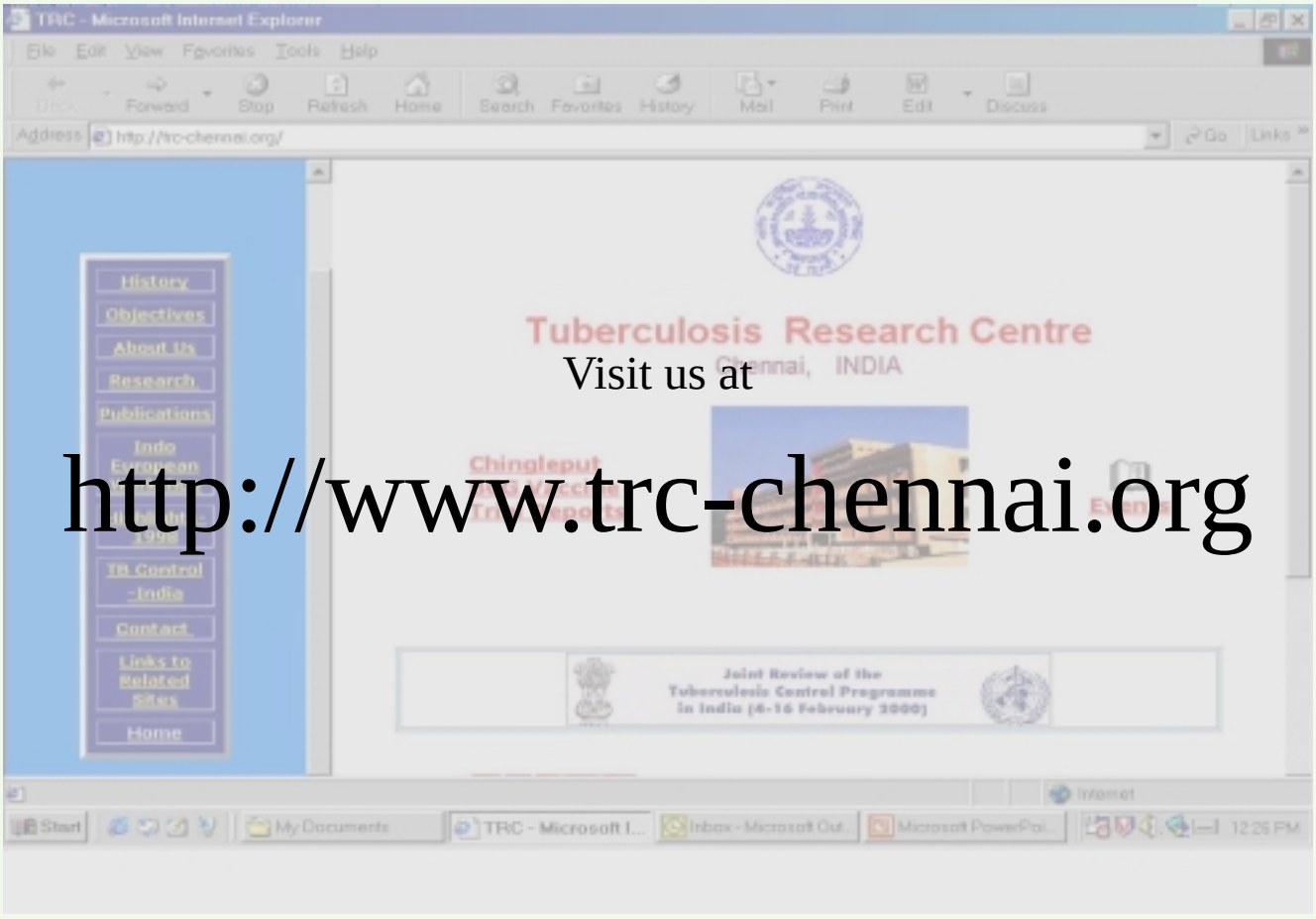
The participants (Professors, Assistant Professors or Readers from the Departments of Medicine or Thoracic Medicine) were drawn from Tamil Nadu, Andhra Pradesh, Kerala and Pondicherry. Presentations were made on the philosophy and structure of the RNTCP with special emphasis on areas of concern to academicians and group work was carried out to develop the strategies and recommendations for integrating RNTCP into the undergraduate and postgraduate teaching programmes and means and methods to promote RNTCP in forums other than classrooms.

World TB day

TRC participated in the World TB day celebrations of Chennai Corporation inaugarated by the Hon'ble Mayor Thiru.M.K.Stalin, MLA

TRC organised a public exhibition on the occasion of World TB day at the centre.

TRC's website launched on World TB day, 1999



Highlights 1999-2000

- **Model DOTS Project inaugurated to assess epidemiological impact of DOTS**
- **Annual Risk of Infection and Disease Surveys initiated in Tiruvallur area**
- **Molecular epidemiological studies to reinforce conventional epidemiology started**
- **Clinical pharmacology unit established as part of an ICMR national network**
- **Division of HIV - AIDS created to focus on HIV/TB related issues**
- **Peripheral clinical research units set up to promote partnerships with city hospitals**

- **Treatment of tuberculosis successfully shortened to 3 to 4 months using ofloxacin**
- **Programme oriented randomized controlled clinical trials yield promising results**
- **Drug resistance surveillance studies highlight low prevalence of resistance to rifampicin**
- **Genes of mycobacteria related to virulence and persistence cloned and expressed**
- **Novel non-HLA polymorphisms linked to tuberculosis**
- **20 immunodiagnostic tests evaluated**

- **Over 2000 medical and para-medical RNTCP programme personnel trained**
- **ACT (Advocacy for Control of Tuberculosis) launched in association with THE HINDU to promote government - private sector mix**
- **Six major international and national workshops held to promote consensus and development of protocols**
- **Web site www.trc-chennai.org hosted to facilitate wider dissemination of research and policies**

DIVISION OF CHEMOTHERAPY

OVERVIEW

The Division of Chemotherapy is the fulcrum around which the Tuberculosis Research Centre revolves. The unit specializes in the conduct of randomized clinical trials in the treatment of tuberculosis. The strength of the Unit is its infrastructure, consisting of physicians, nursing staff, social workers and health visitors, which is ideally geared for carrying out treatment trials in tuberculosis. Over the years it has earned a well-deserved reputation for high quality research. Many landmark research studies in tuberculosis, such as the efficacy of domiciliary treatment, intermittent treatment regimens and short course regimens for pulmonary and extra-pulmonary tuberculosis have been carried out by the unit, with active support from the other departments of TRC. These studies have helped to influence treatment practices for tuberculosis worldwide. After carrying out a large number of clinical trials to establish the scientific foundation for rational treatment regimens, the Unit is now focussing its attention on evolving treatment regimens that are directly relevant to the tuberculosis control programme. Another area of recent activity for the Unit is in the realm of sociological issues in tuberculosis.

NEW INITIATIVE

Model DOTS Project
HIV/AIDS Division

RESEARCH FOCUS

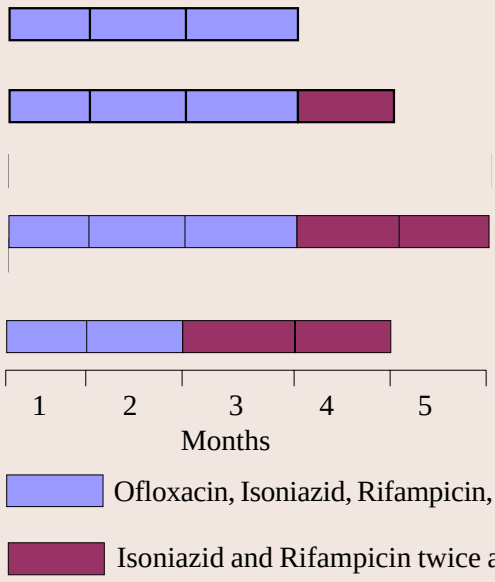
Shortening the duration for treatment of tuberculosis
Tuberculosis control programme oriented clinical trials
Tuberculosis and diabetes mellitus

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

A long felt need for the control of tuberculosis has been a treatment regimen of the shortest possible duration that results in high bacteriological quiescence at the end of treatment, and a low relapse rate during follow up. Based on the results of an *in vitro* study at the centre which demonstrated the bactericidal activity of ofloxacin, the centre is currently undertaking a randomised clinical trial to assess the efficacy of regimens of 3, 4 and 5 months in the treatment of sputum positive pulmonary tuberculosis.

This is a randomised controlled clinical trial being conducted in Chennai and Madurai. Adult patients with newly diagnosed sputum positive pulmonary tuberculosis were randomly allocated to one of the following 4 regimens:

Regimens



Regimen 1: *(ofloxacin, isoniazid, rifampicin and pyrazinamide daily for 3 months)*

Regimen 2: *(same as in Regimen 1, followed by isoniazid and rifampicin twice a week for 1 month)*

Regimen 3: *(same as in Regimen 1, followed by isoniazid and rifampicin twice a week for 2 months)*

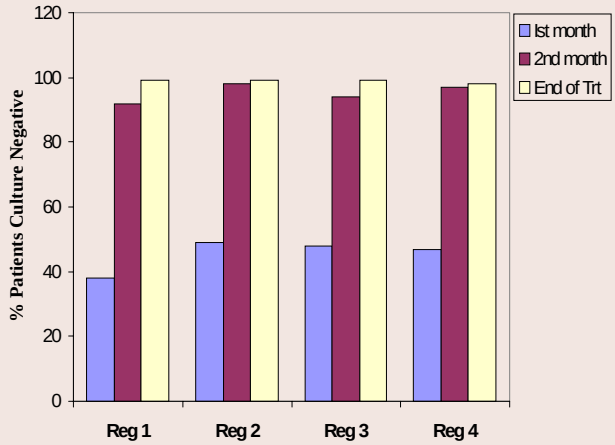
Regimen 4: *(ofloxacin, isoniazid, rifampicin and pyrazinamide daily for 2 months, followed by isoniazid and rifampicin twice a week for 2 months)*

All drugs were given under direct supervision. Patients who defaulted for treatment were visited at home and motivated to attend. Intake to the study was completed in December 1998. It is proposed to follow up the patients for five years.

A total of 529 patients were admitted to the study. After excluding 113 patients, results at the end of treatment are available for 416 patients, 360 with bacilli sensitive to all drugs, and 56 with bacilli resistant to one or more drugs.

The results of sputum culture conversion are shown in Figure1. The proportion of patients becoming culture negative at the end of two months of treatment is a measure of the bactericidal activity of the regimen, and correlates well with relapse rates.

Fig.1 Sputum Culture Conversion

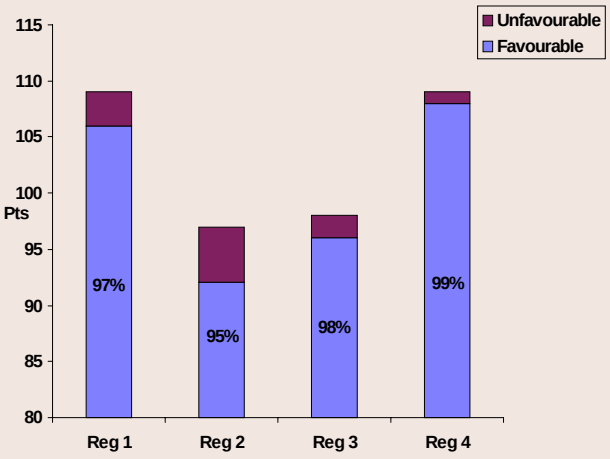


In patients with drug sensitive bacilli the culture negativity at two months was 92%, 98%, 94% and 97% in the regimens 1,2,3 and 4 respectively

Results of the bacteriological status at end of treatment are shown in Figure 2. Forty seven patients had pre -treatment resistance to isoniazid, all but two of whom had a favourable response. Eight patients had resistance to rifampicin and isoniazid, one of whom was also resistant to ofloxacin (MDR Tuberculosis). Of these, five patients had an unfavourable response, one relapsed and the other two had favourable response.

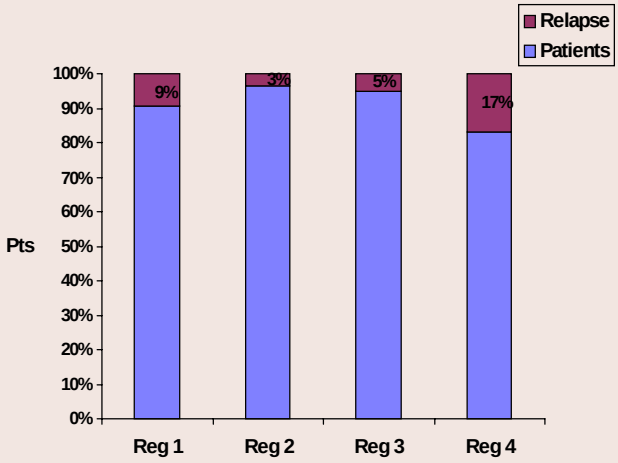
Patients have now been followed up for 20 to 48 months from the start of treatment. Relapse rates after successful completion of treatment is a measure of the sterilising activity of the regimens. The relapse rates after treatment are shown in Figure 3. The differences in the relapse rates between Regimen 2 and Regimen 3 (which each had an intensive phase of three months) compared to Regimen 4 (which had an intensive phase of only two months) was statistically significant (p=0.03).

Fig 2: Bacteriological status at end of treatment



Bacteriological quiescence at the end of treatment was observed in 97% , 95%, 98% & 99 % respectively in the four regimens.

Fig 3: Relapse after treatment



Relapses during follow-up occurred in 9%, 3%, 5% and 17% respectively in the four regimens.

Preliminary results of this study, in which ofloxacin has been used as one of the drugs in primary chemotherapy of tuberculosis for the first time, has shown that all the regimens yielded very good results at the end of treatment with practically all patients showing a favourable response. Relapses were low in the four and five month regimens which had an intensive phase of three months. Relapses were higher in the four month regimen that contained only two months of the intensive phase. Follow up is continuing.

Evaluation of an oral 8 month regimen with a non- rifampicin continuation phase in the treatment of sputum positive pulmonary tuberculosis

The prompt and effective treatment of patients with sputum-positive pulmonary tuberculosis is currently the best available strategy for breaking the chain of transmission of infection in the community. In parts of the world where the disease is endemic as in India, large-scale treatment of patients must be undertaken in order to control tuberculosis.

The currently recommended regimen under the Revised National Tuberculosis Control Programme (RNTCP) in India is of 6 months' duration, thrice weekly throughout, with an intensive phase of 24 doses of 4 drugs, rifampicin, isoniazid, pyrazinamide and ethambutol, followed by 2 drugs, rifampicin and isoniazid for 4 months: 2 RHZE3/4RH3 . Under

programme conditions, the first phase is expected to be given strictly under direct observation of a health provider, either at the treatment centre or at the home/work-place of the patient (DOTS). During the continuation phase however, the patient is expected to receive only one dose under direct observation, while the other two doses for the week are supplied to the patient for self-administration with proper instructions. This procedure opens up the possibility of "concealed irregularity", with patients forgetting to consume some of the doses meant for self-administration. Considering the possibility of irregularity with a rifampicin-containing phase (RH3), it was decided to evaluate a regimen with the same intensive phase, but with 6 months of daily isoniazid and ethambutol given on a once-weekly basis in the continuation phase i.e. 2RHZE3/6HE.

Patients are recruited to the study from among chest symptomatics attending government chest clinics in the cities of Chennai and Madurai. The eligibility criteria for admission to the study are as follows:

- Age 12 years or more
- Prior chemotherapy for less than one month
- Living in Chennai/Madurai within a defined, visitable area
- Likely to remain in this area for at least 2 years
- Will permit home visits
- Willing to attend thrice-weekly for 1st 2 months & once-weekly thereafter during treatment phase, and once a month for follow-up
- Has produced 2 or more sputum smears positive for AFB

Patients thus enrolled are treated with the regimen 2RHZE3/6HE. The dosage schedule is given below:

	Rhythm of administration	
Drug	Thrice-weekly	Daily
Rifampicin	450 mg*	—
Isoniazid	600 mg	300 mg
Pyrazinamide	1500 mg	—
Ethambutol	1200 mg	800 mg

* 600 mg in patients weighing 60 kgs or more.

Before admission in to the study, the clinical and sociological status of the patients are assessed. Routine radiological, bacteriological and biochemical investigations are performed according to the protocol.

During the treatment phase, the patients are followed up at regular monthly intervals and checked for symptoms of drug intolerance or departure from the study regimen. The required radiological and bacteriological tests are also performed.

All subjects are treated on a domicilliary basis. If a patient fails to attend for treatment, his/her home is visited the same afternoon by a health visitor, and on subsequent days by a social worker and a medical officer in that order. These visits are continued until the patient is retrieved. Further action for long-term defaulters is taken for individual patients depending on the reasons for default. Surprise home visits with pill counts are carried out twice a month by a health visitor for patients in the continuation phase of treatment when self-administration of drugs is expected. The progress made by patients is monitored at monthly intervals upto the 24th month after start of treatment.

Definitions of response and relapse

Favourable bacteriological response: All 6 cultures negative during the last 2 months of chemotherapy.

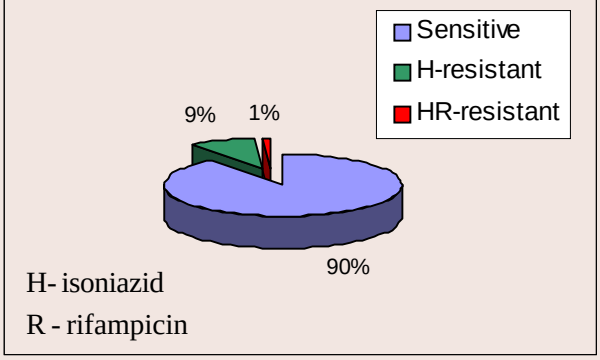
Unfavourable bacteriological response: Two or more cultures positive in the last 2 months of treatment, including atleast 1 in the last month, with atleast 1 culture growing 20 colonies or more. Patients who had their treatment changed for persistent bacteriological positivity or radiographic and/or clinical deterioration, and those who died of tuberculosis during the treatment phase are also classified as having an unfavourable response.

Bacteriological relapse requiring treatment: Two or more cultures positive for *M.tuberculosis* in a 2 month period, at least 1 of which has a growth of 20 colonies or more, and associated with a positive smear.

Intake to the study commenced in December 1998, and results at the end of treatment for 200 patients are reported here.

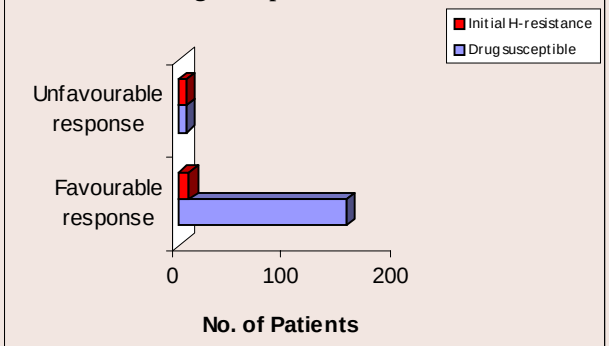
Of the 200 patients admitted to the study, 20 were excluded from this analysis for various reasons. The initial drug susceptibility pattern of the patients is given in Figure 1. 90% had bacilli susceptible to all anti-tuberculosis drugs, while 9% were resistant to isoniazid and 1% to isoniazid and rifampicin. Response at the end of treatment is shown in Figure 2, for drug-susceptible and isoniazid-resistant groups. The lone patient with MDR-TB had a doubtful response at the end of chemotherapy.

Fig.1 Initial drug susceptibility pattern



Of total of 180 patients in analysis,162 had bacilli sensitive to all anti-tuberculosis drugs, while 17 were resistant to isoniazid and 1 to rifampicin as well.

Fig.2 Response to treatment



Of the 162 susceptible cases, 94% responded favourably to treatment, while 4% showed an unfavourable response. Of 17 isoniazid-resistant cases, 10 responded favourably and 7 had unfavourable response.

All patients are being followed up for a period of 2 years from the commencement of chemotherapy. The study is in progress.

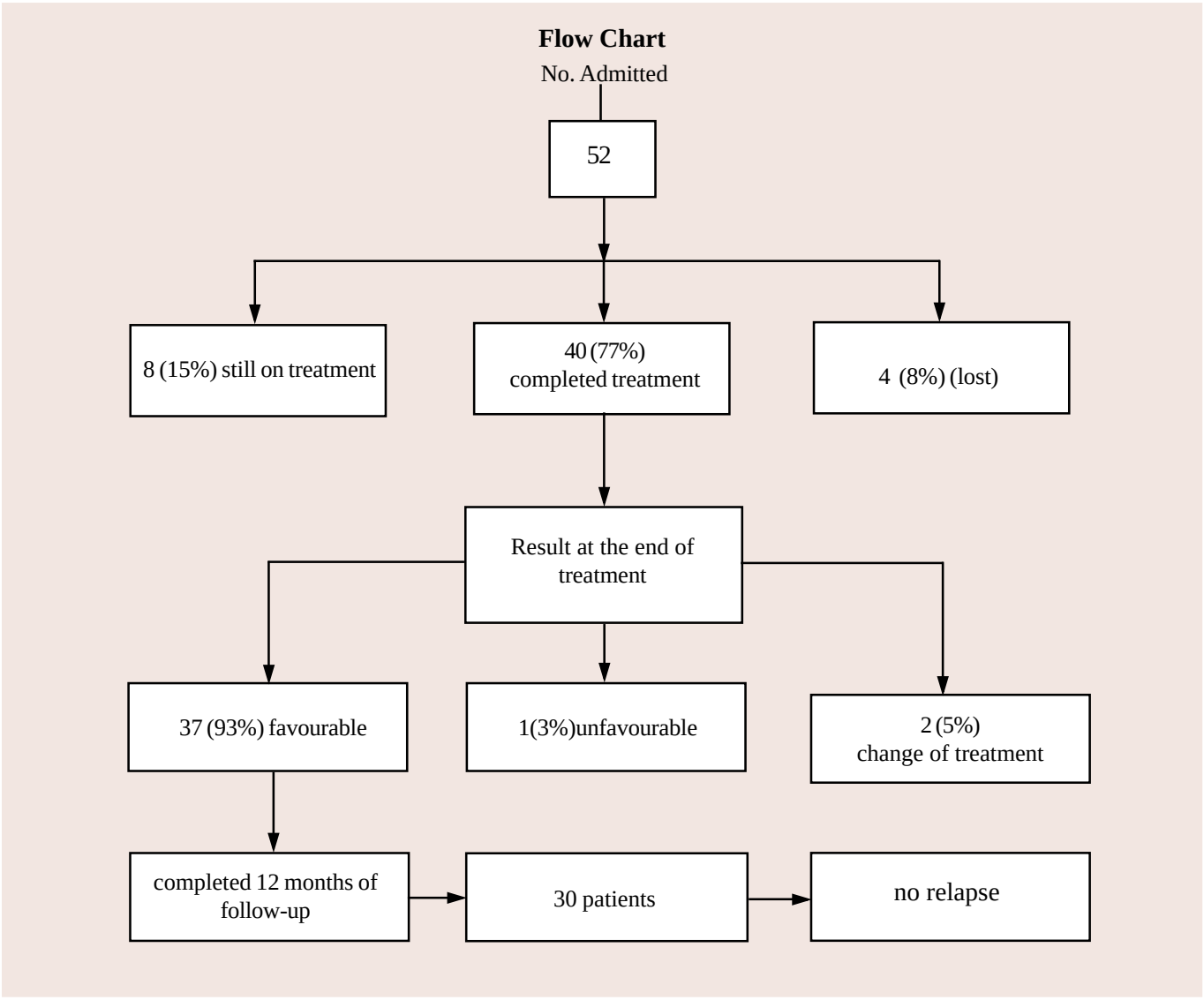
A study of category I regimen (RNTCP) for treatment of pulmonary tuberculosis associated with non-insulin dependent diabetes

Under Revised National TB Control Programme, category I regimen ($2EHRZ_3/4RH_3$) is recommended for patients with diabetes mellitus. Recent surveys in India indicate that the prevalence of tuberculosis among symptomatics varies from 4.4 to 11.0 per 1000 and it is rising from 10% to 25% by the age of 60 years as per a WHO report. Similarly, non-insulin dependent diabetes (NIDDM) is also more common among older subjects. It has been reported from Japan (retrospective analysis) that a 12-month "short-course chemotherapeutic" regimen was effective in diabetic patients with pulmonary tuberculosis. It is proposed to assess the efficacy of the category I regimen, in relation to both cure and relapse rates during a period of 3 years from admission in to the study.

Patients attending the Government General Hospital, Kilpauk Medical College Hospital, Institute of Thoracic Medicine and the TRC suspected to have both pulmonary tuberculosis and NIDDM will be investigated at the centre. It is proposed to admit 60 patients to the study.

All patients will receive the following regimen: ($2EHRZ_3/4RH_3$)-Ethambutol 1200 mg plus isoniazid 600 mg with pyridoxine 10 mg plus rifampicin 450 mg for patients weighing less than 60 kg and 600 mg for patients weighing 60 kg or more plus pyrazinamide 1.5g for all patients three times a week under supervision for 2 months followed by rifampicin plus isoniazid in the same dose for the next 4 months.

A total of 52 patients have been admitted so far (Flow chart). There were 43 (83%) males. The mean age was 49 years (range: 34-70 yrs.) and the mean body weight was 48.4 kg. (range: 32.6-60.0 kg.).



Among the 52 patients, 8 are still on treatment and 4 (7.7%) were lost to treatment as they all migrated out of Chennai. Of the remaining 40 patients, 37 (93%) became culture negative at the end of treatment. Two patients had change of treatment due to drug toxicity. One patient had an unfavourable bacteriological response as he had developed resistance to isoniazid alone after taking treatment. Of them, 30 have completed 12 months of follow-up after treatment and none have relapsed.

The smear and culture status related to response of treatment in 38 patients is described in the adjoining table. The proportion of patients who became smear negative at each month were 37%, 66%, 79%, 87%, 92% and 89% respectively. The patient who had an unfavourable response became smear and culture negative by2nd month but became smear positive by 3rd month and culture positive by 5th month and remained positive subsequently.

Smear and Culture status

Month	S+	C+	S_O	C+	S+	C_O	S_O	C_O
	No.	Failure	No.	Failure	No.	Failure	No.	Failure
1	19	1	2		4		12	
2	3				9		25	1
3					7	1	30	
4					4		33	1
5		1			2		35	
6		1	1		2		34	

S - Smear C – Culture + - Positive 0 - Negative

Low rate of emergence of drug resistance in sputum positive patients treated with short course chemotherapy

Recently concerns have been raised about the possible "amplification" of drug resistance in patients who have organisms resistant to one of the two drugs used in the continuation phase, especially in areas with a high level of initial drug resistance. This concern applies particularly to patients with isoniazid resistance; if these patients develop resistance to rifampicin as well, their treatment will be difficult and the likelihood of cure will be much lower. In order to examine the issue of emergence of resistance among patients treated with the currently used drugs in the programme, we examined the data of 2 studies in which the 4 drugs recommended in the programme were used.We report response to treatment, relapse, and emergence of drug resistance among patients treated in these controlled trials.

A retrospective analysis of studies using the following regimens was undertaken:

(1) 2 $HRZE_7$ / 6 HE_7 (2) 2 $HRZE_2$ / 4 HRE_2

(3) 2 $HRZE_3$ / 4 HR_2 (4) 3 $HRZE_3$ / 3 HR_2

Smear and culture examinations were carried out on four sputum specimens pre-treatment, three during treatment and two each at monthly intervals during the follow up period of 24 months. Drug susceptibility testing was done on 2 positive pre-treatment cultures and on any subsequent positive culture. An unfavourable response was defined as death due to tuberculosis, clinical and/or radiographic deterioration or persistent sputum positivity requiring a change of treatment, or 2 or more positive cultures during the last 2 months of treatment. Relapses were bacteriologically confirmed.

Of the 1817 patients studied, 1435(79%) had organisms susceptible to all drugs. Of the 1435 patients with initially susceptible isolates, 25(2%) had unfavourable response and 95 of 1356 (7%) had a bacteriological relapse. 12(1%) developed resistance to isoniazid or rifampicin or both. Of 320 patients who had isoniazid resistance prior to treatment, 60 (19%) had unfavourable response and 31 of 247 (13%) had relapsed, 34 (11%) had emergence of resistance to rifampicin (32 of 60 with unfavourable bacteriologic response and 2 of 31 who had relapse). The outcomes of patients who received a three-drug continuation phase (isoniazid, rifampicin, and ethambutol) were no better than outcomes of patients who received a two-drug continuation phase. Data on resistance to ethambutol and pyrazinamide is not available.

In a population of patients with a high rate of 21% pre-treatment isoniazid resistance, the emergence of resistance to rifampicin occurred among only 2% of patients treated with standardized short-course treatment. Sputum positive pulmonary tuberculosis patients can be successfully treated with standardized short-course chemotherapy regimens.

Status of long absentees among multi-bacillary leprosy patients admitted to a controlled clinical study

Two hundred and ten lepromatous leprosy patients with an average BI of 2.5, were admitted to a controlled clinical trial of two multidrug regimens and were followed up for 20 years. The occurrence of relapse even among the regular cases prompted us to under take a one time assessment of long absentees, with the aim to find out their clinical and bacteriological status.57 patients were excluded from analysis at 120 months for various reasons. Of these, 33 patients were identified as long absentees and were the subject of one time assessment. Of the 33 patients identified, 21 were retrieved. The main reason for default was migration. The patients had defaulted at various stages of treatment and follow-up. Majority of patients had defaulted beyond 3 years.At the time of default, 90% had marked to moderate clinical improvement though majority of them were bacteriologically positive,(BI 0.00 to 4.17). It is an encouraging information as it justifies the use of Fixed dose therapy of 24 months in the programme and paves the way for achieving the goal of elimination of leprosy.

Staff List

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Meenalochani Dilip, M.A.
Chandra Suresh, M.A.
D.Kalaiselvi, M.A.
P.Murugesan, M.A.
C.T.Rajasekara Sastry

Capacity Building

Dr.Ranjani Ramachandran - M.D.(Microbiology) - The Tamil Nadu Dr.M.G.R. Medical University.

Dr.D.Bhaskaran - DTCD - The Tamil Nadu Dr.M.G.R. Medical University.

Doctor of Philosophy

Thesis in progress

Mrs.Beena Thomas - Gender differentials in response to HIV - University of Madras.

Radiographer course

Mr.T.Gowri Shankar - Institute of Radiology and Oncology, Chennai (Completed)

Mr.E.Kirubakaran - Institute of Radiology and Oncology, Chennai (In progress)

Introductory Course on Epidemiology and Biostatistics held at Sundaram Medical Foundation, Chennai during Sept.1999 - **Mrs.Beena Thomas**

Master's Training Programme on 'Arivoli Iyyakkam' organized by Chennai Corporation held at Chennai during Oct.1999. - **Dr.Geetha Ramani Shanmugam & Mrs.Mohanarani Suhadev**

Arivoli Iyyakkam Training for Volunteers conducted by Chennai Corporation during Dec.1999
- Dr.Geetharamani Shanmugam and Mrs.Mohanarani Suhadev

Training programme on Holistic approach to HIV/ AIDS conducted by Christian Counselling Centre held at CMC, Bagayam, Vellore - **Mrs.Mohanarani Suhadev & Mrs.Chandra Suresh** (July-Aug.2000) **Mrs.Beena Thomas & Meenalochani Dilip** (Nov. 2000)

Participation in Conferences / Workshops / Symposia

National

Seminar on Intersectoral Partnership in Health Care held at Madras School of Social Work, Chennai during Feb.1999 - **Mrs.Beena Thomas**

ICMR-WHO Workshop on 'Ethical Issues in Biomedical Research' held at New Delhi during Mar. 1999 - "Ethical Issues in Clinical Trials"-Faculty
- Dr.Rema Mathew

Symposium on 'Tuberculosis' organised by Astra Zeneca Ltd.held at Bangalore during June 1999
- "Patient Recruitment to Clinical Trials"
- Dr.M.S.Jawahar

Southern Regional Conference of Pharmacologists held at Perundurai during June 1999 - "Multi Drug Resistant Tuberculosis"- **Dr.M.S.Jawahar**

Prevention and Spread of rumours in STD/HIV AIDS Control Programme, Family Welfare Training Centre held at Chennai during June 1999
- Mrs.Sudha Ganapathy

7th Annual International 'CME 99' organised by MediCiti Hospitals held at Hyderabad during Aug.1999 - "Multi Drug Resistant Tuberculosis - the Indian Scenario" - **Dr.M.S.Jawahar**

ICMR-WHO National Workshop on 'Health Research Management' organised by the TRC held at Chennai during Sept.1999 -"Ethical Aspects of Health Research Initiatives" - Lead Discussant
- Dr.Rema Mathew

Workshop on 'Involvement of Private Practitioners in the RNTCP' organised by the Tuberculosis Research Centre and the Ministry of Health, Govt. of India held at Chennai during Nov.1999 - **Dr.Usha Ramanathan & Dr.M.S.Jawahar**

54th National Conference on TB & Chest Diseases held at Patna during Dec.1999 -**Mrs.Mohanarani Suhadev** (Perception and Attitude of Pulmonary Tuberculosis Patients on treatment from Private & Public Sector), **Mrs.Meenalochani Dilip** (Women and Tuberculosis), **Mrs.K.Jagga Rajamma** (Treatment Compliance of Tuberculosis patients who were treated with regimens of their choice)

Workshop on HIV/AIDS for HIV positive women organized by NSS Unit of Madras School of Social Work held at Chennai, Feb.2000
- Mrs.Beena Thomas

Symposium on 'Status of Tuberculosis at 2000' organised by the Sir Dorabji Tata Centre for Research in Tropical Diseases, Microbiology and Cell Biology Dept., Indian Institute of Science held at Bangalore during Mar.2000 - **Dr.M.S.Jawahar** (The emerging threat of Multi Drug Resistant Tuberculosis), **Dr. Rema Mathew** (Childhood Tuberculosis : Complexities of Diagnosis, Management and Prevention)**, Mrs.Beena Thomas** (Tuberculosis in Women)

Role of NGOs in RNTCP organized by TNVHA and TRC held at TRC during Mar. 2000
- Mrs.Sudha Ganapathy, Dr.Geetharamani Shanmugam & Mrs.Beena Thomas

Workshop on 'Involvement of NGOs in RNTCP' organised by Tamil Nadu Voluntary Health Association held at Chennai during Mar.2000
- Dr.Usha Ramanathan

Regional conference on 'Health Promotion & Education – Millennium Challenges' by IUHE South East Asia Region held at Mysore during Apr.2000
- Dr.Usha Ramanathan & Mrs.Beena Thomas

Workshop on 'Ethics in Social Sciences and Health Research' held at the Indian Institute of Technology, Chennai during Apr.2000 - **Dr.Rema Mathew**

Workshop on 'Role of media, financial and welfare organisations among positive persons' organised by TANSACS and Women & AIDS committee held at Chennai during Apr.2000 - "Psychological problems

of positive persons" - **Mrs. Meenalochani Dilip** Participants -**Mrs.Beena Thomas** & **Mrs.Sudha Ganapathy**

Symposium on 'Clinical Research in India – the Road Ahead' organized by the All India Institute of Medical Sciences in association with the Indian Council of Medical Research held at New Delhi during Sept.2000 - **Dr.M.S.Jawahar**

Seminar on 'Pen your mind down-challenging mental health' organised by BANYAN held at USIS, Chennai during Oct.2000 - **Mrs.Beena Thomas**

Seminar on 'Human rights' organised by Dept. of Social work, Madras Christian College held at Chennai during Oct.2000 - **Dr.Geetharamani Shanmugam**

Workshoop on family health-RTI/STD/HIV/AIDS conducted by Tamil Nadu State AIDS cell and committee on Women and AIDS held at Chennai during Oct.2000 - **Mrs.Meenalochani Dilip**

NAPCON 2000 organised by the Indian Chest Society and the National College of Chest Physicians and Satellite Symposium on Tuberculosis held at Kanpur and Kajuraho during Nov.2000 -"Multi Drug Resistant Tuberculosis – the current scenario" - **Dr.M.S.Jawahar**

Seminar on HIV/AIDS organised by World Vision on World AIDS day held at Chennai during Dec.2000 - "Our response to HIV/AIDS" - **Mrs.Beena Thomas**

Indo French Workshop on 'Tuberculosis' held at Chennai during Dec.2000 - **Dr.Usha Ramanathan**

Curriculum Development Workshop for Course on 'Modern Approaches to Epidemiology and Control of Vector Borne Diseases' organized by the Vector Control Research Centre, Pondicherry and the Wellcome Trust Centre for the Epidemiology of Infectious Diseases, University of Oxford held at Pondicherry during Dec.2000 - **Dr.M.S.Jawahar**

55th National Conference on TB & Chest Diseases held at Calcutta during Dec.2000 - "Socio economic impact of parental TB on children" - **Dr.Geetharamani Shanmugam** Participants - **Mrs.Sudha Ganapathy, Mrs.Nirupa Charles & Mrs.Sheela Fredricks**

Conference organized by Apollo Hospitals, Hyderabad and American Telugu Association held at Hyderabad during Dec.2000 - "Clinical Perspectives of Multi Drug Resistant Tuberculosis" - **Dr.M.S.Jawahar**

International

11th International Symposium of IUCISD -"Social Development for the New Millennium: Visions & Strategies for Global Transformation" held at University of Cape Town, South Africa during July 1999 -"Global Health Emergencies – A threat to social development in the New Millennium – Need for people oriented control strategies" - **Mrs.Beena Thomas**

Symposium on 'Infectious Diseases: Past Lessons for Future Directions' at the Annual Academic Sessions of the Sri Lanka College of Microbiologists held at Colombo, Sri Lanka during June 2000 -"Multi Drug Resistant Tuberculosis – the Asian experience" - **Dr.M.S.Jawahar**

Protocol Development Workshop for WHO Multicentric Collaborative Project on 'Gender Differentials in Tuberculosis Control' held at Geneva during Dec.2000 - **Dr.M.S.Jawahar** (Principal Investigator)

Membership in Expert Committees & Special Assignments

Member of Technical Research Group on Research and Development on HIV/AIDS constituted by the National AIDS Control Organisation (NACO), at Pune, Apr.1999 - **Dr.M.S.Jawahar**

Member of Special Panel constituted by Ministry of Health, Govt. of India, to evaluate Master's Degree in Public Health (MPH) of the Sree Chitra Tirunal Institute of Medical Sciences and Technology, Thiruvananthapuram, May 1999 - **Dr.M.S.Jawahar**

Organiser & Faculty Person of the ICMR-WHO National Workshop on Revised National Tuberculosis Control Programme for Medical College Faculty held at Tuberculosis Research Centre, Chennai during Oct.2000 - **Dr.M.S.Jawahar**

Faculty Person for the ICMR-Ministry of Health National Protocol Development Workshop on HIV-TB held at Chennai during Nov.2000 - **Dr.M.S.Jawahar**

External Examiner for Viva Voce (Field Work) for M.A.

(Social Work) students of Madras Christian College, Tambaram during Oct.2000 - **Mrs.Sudha Ganapathy**

Member of the membership committee of the Inter-University Consortium for International Social Development(IUCISD) - **Mrs.Beena Thomas**

Advocacy

CME Programmes for Professors and Asst. Professors of Medical Colleges, at Madras Stanley and Kilpauk Medical Colleges held at Chennai during Aug.1999 -"Global Burden of Tuberculosis" - **Dr.M.S.Jawahar**

Panel Discussion on the RNTCP organised by the Indian Medical Association, Ambattur Branch, and Advocacy for Tuberculosis Control (ACT) held at Chennai during Aug.1999 - **Dr.M.S.Jawahar**

Meeting of the Indian Medical Association, Nilgris branch held at Coonoor during Nov.1999 -"The Challenge of Tuberculosis in the New Millennium" - **Dr.M.S.Jawahar**

Role of IEC in Scientific Communication held at NIN, Hyderabad during Dec.1999 -"IEC activities at TRC" - **Dr.Geetharamani Shanmugam**

CME -'Forum 2000', for practising physicians organised by Sundaram Medical Foundation held at Chennai during Feb.2000 -"MDR-TB – the responsibilities of the General Practitioner" - **Dr.M.S.Jawahar**

Consultation programme on 'Socio economic development programmes for Adidravidar Community' organised by TAHDCO held at Chennai during June 2000 - **Dr.Geetharamani Shanmugam**

Awareness programme on Tuberculosis organised by Family Life Institute of Madras Christian College, Tambaram during Sept.2000 - **Dr.Geetharamani Shanmugam & Mrs.Meenalochani Dilip** (Resource Persons)

Training for Health Inspectors on RNTCP organised by Family Welfare Institute held at Chennai during Sept.2000 - "Social aspects of Tuberculosis" - **Mrs.Sudha Ganapathy**

Residential, informal training for Transit School Teachers organised by the Tamil Nadu Slum Clearance Board held at Chennai during Sept.-Oct.2000 - "Social aspects of Tuberculosis" - **Dr.Geetharamani Shanmugam** (Resource Person)

Indo-US (ICMR – UCLA) training program for investigators in the "Study of sexual and psychological health of HIV positive and negative women in India" held at Chennai during Oct.2000 - **Mrs.Beena Thomas, Mrs.Meenalochani Dilip & Mrs.Mohanarani Suhadev**

'Orunginaippu' Programme of Corporation of Chennai with Private Sector participation in the Eradication of Communicable Health Problems - a CME Programme on 'the Role of Private Practitioners in Tuberculosis Control' held at Chennai during Dec.2000 - "Overview of Tuberculosis Situation in India and an introduction to RNTCP" - **Dr. M.S.Jawahar**

IEC Activities:

The Model DOTS Project was launched on March 24th (World Tuberculosis Day),1999 at Thiruvallur. An exhibition on Tuberculosis was organized on this occasion. Social workers from the centre took active part in planning,organizing and conducting the exhibition. A large number of school students and public visited the exhibition. The Ministers for Health & Electricity and Dairy Development and Health Secretary, Govt. of Tamil Nadu and the Collector of Thiruvallur District and other dignitaries graced the occasion. A tamil video film on tuberculosis "Kanne Kanmaniye" was released on this occasion.

An exhibition was organized at the centre on 24th March 1999 for the benefit of patients. Illustrations and messages in Tamil and English on tuberculosis were displayed and released.

The Tamil Nadu Chief Minister's Comprehensive Health Care Scheme was inaugurated at Padianallur, Thiruvallur District on 29th November1999. The centre along with the District Tuberculosis centre had put up a stall on tuberculosis at the exhibition organized on this occasion. Public, students and representatives from NGOs visited the exhibition.

Contact Persons

Design and conduct of randomised clinical trials
Gender & Tuberculosis
MDR-TB
Tuberculous lymphadenitis
Training Programmes for medical & paramedical staff students
Dr.M.S.Jawahar
trcicmr@md3.vsnl.net.in(clinic1)

Biomedical ethics
Programme based randomised clinical trials
Dr.Rema Mathew
trcicmr@md3.vsnl.net.in(clinic2)

Public private mix in TB control
Dr.R.Balambal
trcicmr@md3.vsnl.net.in(clinic2)

Patient management in randomised clinical trials
Dr.K.Rajaram
Dr.Paulin Joseph
Dr.Usha Ramanathan
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Sociological aspects of tuberculosis
Mrs.Sudha Ganapathy
trcicmr@md3.vsnl.net.in(clinic2)

OPERATIONAL RESEARCH

Establishment of Model DOTS Centre for Implementation for Training and Research in TB control

Background

Directly Observed Treatment-Short course (DOTS) is universally accepted as the treatment strategy for tuberculosis control in both developing and developed countries. Government of India is implementing this strategy in the Revised National Tuberculosis Control Programme (RNTCP) in a phased manner since 1993. However, the epidemiological impact of this strategy on the tuberculosis problem in a community has not been studied. The Tuberculosis Research Centre (TRC) has undertaken a project in collaboration with the Government of Tamil Nadu to establish Model DOTS center for DOTS implementation, Tuberculosis controls, training and research to examine the epidemiological impact of DOTS. The project is undertaken with the technical support from WHO and financial support from USAID. It is proposed to measure the epidemiological impact of this strategy in this community over a period of at least10 years.

Objectives of the project

1. Establish a Model Centre for DOTS implementation in a population of approximately 5 lakhs in Tiruvallur district in Tamil Nadu, India.
2. Establish a Centre for DOTS demonstration and training.
3. Strengthen the infrastructure at state level, particularly in the districts surrounding the project area.
4. Assess epidemiological impact of DOTS using ARI (tuberculin surveys), Disease survey, RFLP and Drug resistance surveys.
5. Conduct operational research on key aspects of the DOTS strategy.

Project Area

Five Panchayat unions in Tiruvallur District covering a population of 5,87,000 were selected for Model DOTS project (see attached map). There are 218 villages in these blocks. This is the same area where the famous "Chingleput BCG Study" was conducted. The advantage of availability of information of 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 of time the epidemiological impact of DOTS programme can effectively be measured here.

DOTS implementation

After completing the necessary civil works for microscopy centres and training the medical and paramedical staff in the area, the service delivery was initiated on May 3rd 1999. 1150 patients have been admitted to treatment as of 30 September 2000.

DOTS demonstration and training

Medical and paramedical trainees are taken regularly to the area for field level training in RNTCP. So far 231 medical officers (MO Trainers) including, 13 WHO-RNTCP Consultants, 182 Senior Treatment Supervisors, 77 STLS and 28 Laboratory assistants have been given field training in the area. The training facilities are being upgraded and it is hoped that further training will be undertaken in Tiruvallur itself.

State strengthening

Tuberculosis Research Centre is providing all assistance as required by the state level officers for Control activities. We are involved in training of the trainers from various districts in Tamil Nadu. So far 195 MO trainers, 91 MOs 182 STS, 77 STLS and 28 LTs and 34 pharmacists have been trained. We have helped in the procurement of two wheelers for the STS and STLS for the programme. We are in close contact with the Chennai corporation area where the programme is implemented and provide all technical assistance as and when required.

Due to turn over of the staff, training of MO trainers is continuing. Training for STS has been completed for Tamil Nadu. STLS training is still ongoing. This was due to the delay in identifying the STLS by the state government.

In addition, the center has undertaken training for the district level medical officers of Bihar, Assam, Manipur and Orissa. We have also given training to the RNTCP consultants appointed by WHO, Medical Officers and Policy makers from Orissa.

Additional aspects of the training

During the training process, we realized the need to include Module 7 to the medical officers (5 day training) and Module 9 to the STS. This has facilitated their understanding on reporting system, programme indicators for medical officers and the logistics of drugs and consumables for the STS.

In order to facilitate better understanding of the definitions used in RNTCP with regards to the Type of cases and Treatment outcome, we have introduced a system of role-plays for each of the definitions and various practical problems encountered. Role-plays are used to improve the understanding of motivation and defaulter retrieval procedures.

A question bank has been developed for each of the modules. After each module, one of the participants is asked to summarise the important aspects in the module. This is followed by the question answer session.

After the field training, the participants are asked to summarise their observations and discuss the problems identified with suggestions for solution.

Assessment of epidemiological impact

To assess the epidemiological impact of DOTS implementation, a baseline survey to estimate the prevalence of tuberculosis disease and infection is in progress in the project area. The sample size estimated is 82,000 persons 15 years and above. A random sample of panchayats from the rural area and blocks from the urban area has been selected to provide the required sample size. The survey will be repeated once in three years.

A house-to-house census is being carried out and all individuals in the households are registered. Informed consent is being obtained from all individuals in carrying out the examinations. All individuals 15 years and above are being questioned for symptoms relating to tuberculosis and have a chest X-ray (MMR). All persons are tuberculin tested using 1TU of PPD RT23. Two specimens of sputum (one spot and one overnight) will be collected from all those individuals who are symptomatic or X-ray abnormal or both.

These specimens will be transported to TRC laboratory for smear microscopy by fluorescent method, culture examination and susceptibility testing. Treatment is according to RNTCP procedures under Model DOTS. The patients referred for treatment based on three specimens will be motivated for treatment to the nearest PHC.

Tuberculin survey

Annual Risk of Infection is being estimated by studying 45,000 children below 15 years. A representative sample of panchayats from each one of the five blocks has beeen selected for each survey. The sample size will remain the same for all the surveys but the study sample will be mutually exclusive between any two surveys in each one of the blocks. The survey methodology involves the enumeration of the study population, identification of the children aged 2 months to 14 years, BCG scar reading, tuberculin testing with 1TU RT23 and reading the reaction size 72 to 96 hours after testing. Of the 17,788 children 38% had a BCG scar.

Drug resistance surveillance in MDP area

In order to study the drug susceptibility pattern among patients admitted to treatment in the project area and to study the profile over a period of time to study 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. Sputum specimens have been collected from 1060 patients so far.

Quality assurance

The TRC is providing quality assurance to the programme in the Model DOTS area. Quality control of the sputum microscopy is being done in 2 stages. The STLS during his visits to the microscopy centre, checks all the positive slides and 10% of the negative slides. Slides checked by the STLS and some additional slides are transported to TRC where they are randomized and checked by a senior technician. Thus a quality check is done for the LT of the PHI and for the STLS.

The table below gives the level of agreement between the various readers. The agreement between the readers has been 96% to 99.8%.

Table: Level of agreement of smear microscopy.

	Round I	Round II
PHI-LT vs STLS	312/317(98.4%)	519/520(99.8%)
PHI-LT vs TRC-LT	515/526(97.9%)	407/417(97.6%)
STLS vs TRC-LT	226/235(96.2%)	219/228(96.1%)

Molecular epidemiology

TRC is undertaking RFLP studies on positive cultures obtained from patients started on treatment and those isolated in the survey.

M.tuberculosis clinical isolates were cultivated on LJ medium, harvested and killed at 80°C for 30 minutes. Genomic DNA was isolated and Pvu II-IS6110 RFLP analysis was performed according to standardized methods using a 245 bp right-sided probe. In parallel Alu I cleaved genomic DNA was probed with repetitive DR element isolated from *M.bovis* BCG according to standard protocols.

A total of 500 clinically proven cases of tuberculosis were identified for molecular epidemiological studies from Model DOTS area. Clinical isolates from these cases were grown on LJ medium for 4 to 6 weeks then harvested in TE buffer and killed at 80°C for 30 minutes. Genomic DNA was isolated as per standard method and stored at –20°C in duplicates. Out of these 257 isolates were processed for RFLP using 245 base pair right-sided probe of IS6110. Approximately 1 mg of genomic DNA from each isolate was digested with Pvu II, electrophoresed and southern transferred. The blots were then probed with ECL labeled IS6110 probe as per the protocol given by Amersham labeling kit.

Gender issues in tuberculosis

Data collected from the epidemiological survey is being examined to identify gender differences with respect to infection and disease in the community. Data on cases identified in the MDP area will help to understand the self-reporting and diagnosis of the disease in service programme.

Acceptability of DOTS and DOTS providers

All patients started on treatment are visited by the medical social workers to collect information on the patient's perspective about DOTS and their reactions on the providers. Attempts are also being made to contact the DOTS providers.

Role of bronchodilators in improving the yield of sputum positivity

Some amount of bronchospasm is associated with infection and this may interfere with the quality of the sputum specimen given by the chest symptomatics. A study is being initiated to find out to what extent this problem exists and whether administration of bronchodilators can increase the yield of positivity. The study is being done in collaboration with the Corporation of Chennai in one of its microscopy centres. So far 35 patients have been admitted to this ongoing study.

Deposit method for sputum examination

In order to facilitate smear microscopy at the PHIs for the laboratory technicians, a simple deposit method of smear examination is being investigated in the Tiruvallur MDP area.

A total of 534 sputum samples were collected from 230 pulmonary tuberculosis (PT) patients attending the Govt.Hospital, Thiruvallur and Primary Health Centre, Periyapalayam, where Revised National Tuberculosis Control Programme (RNTCP) is being implemented. The samples were subjected to direct smear (DS) examination for AFB by the Ziehl Neelsen method. The smears were read by the laboratory technicians at the respective centres and reported the results as per the RNTCP guidelines. The samples received on Mondays, Wednesdays and Fridays were gently mixed with 5 to 10 ml of sodium hydroxide-ammonium sulphate (SAS) reagent. The sputum samples received on Tuesdays, Thursdays and Saturdays were treated with phenol-ammonium sulphate (PAS) reagent. The samples were occasionally shaken and left at the room temperature over night or till they were taken up for sediment smear (SS) preparation. The contents of the sputum sedimented after an overnight storage. After decanting the clear supernatant, a drop of the sediment was smeared over a clean glass slide. The smear was air-dried, heat fixed, stained by the Ziehl Neelsen method and read at Tuberculosis Research Centre, Chennai, by proficient readers who were unaware of the DS results.

Dispensing patterns of anti-TB drugs by private pharmacies

A study was undertaken to evaluate the dispensing patterns of anti-TB drugs in an area where RNTCP is implemented and in another area where it is not implemented. The objectives of this exercise were to find out the impact on sales of anti-TB drugs by private pharmacies after implementation of RNTCP in Govt. Health facilities, to assess the knowledge on RNTCP and DOTS of the Private Pharmacists, their dispensing patterns, their purchasing patterns and preference of anti-TB drugs by the patients.

Using a semi-structured, pre-coded interview schedule 100 randomly selected pharmacies in Chennai corporation, all the pharmacies (50) in a rural area where RNTCP is implemented and 150 pharmacies from another area where RNTCP is not implemented were contacted by the TRC staff.

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.
S.Radhakrishnan, B.A. (STS).
E.Prabhakaran,D.M.L.T (STLS)
V.S.Sukumaran, M.A.
M.Kalyanaraghavan, M.Sc.

Participation in Conferences / Workshops / Symposia

National

National consensus meeting on drug resistance organized by Lupin, India held at Chennai during Jan.1999 - **Dr.T.Santha Devi**

Applying research to RNTCP: an informed approach organized by DFID, India held at NTI, Bangalore during Apr.1999 - **Dr.T.Santha Devi**

7th Tamil Nadu Conference on Tuberculosis and chest diseases held at Nagercoil during May 1999 - "Impact of TB on Private for profit providers"
- **Dr.AleyammaThomas**

Symposium on "Tuberculosis – The Indian Perspective" organized by Astra India held at Bangalore during June 1999 - **Dr.T.Santha Devi**

Midterm review of RNTCP: review of central institutes held at Delhi during Sept. 2000 - "Contributions of TRC to tuberculosis programme"
- **Dr.T.Santha Devi**

21st Biennial Conference of IAL held at Chandigarh during Sept.1999 - "Control clinical trial of 2MDT regimens in highly bacilliferous BL/LL leprosy cases - reports on 20 years followup"
- **Dr.AleyammaThomas**

National Conference on 'Quality assurance in Health Care' held at Calcutta during Nov.1999. - "Revised National TB Control Programme in India"
- Dr.Rani Balasubramanian

26th Conference on Tuberculosis and Lung Disease organized by Andhra Pradesh TB association held at Hyderabad during Nov.1999 - "Chemotherapy of tuberculosis challenges and solutions"
- **Dr.T.Santha Devi**

Workshop on 'Involvement of Private Sector in RNTCP' held at Chennai during Nov.1999 - "Briefing on RNTCP with reference to role of private sector"
- **Dr.T.Santha Devi**
Participant - **Dr.Rani Balasubramanian**

Workshop on 'Involvement of Private Practitioners in RNTCP' held at Chennai during Nov.1999
- **Dr.Rani Balasubramanian**

International

30th IUATLD Conference on Lung Health held at Madrid, Spain during Sept.1999 - "Low rate of emergence of drug resistance in sputum positive patients treated with short course chemotherapy"
- **Dr.T.Santha Devi**

Symposium on 'Power, Poverty and TB' organised by tb.net 2000 held at Kathmandu, Nepal during Feb.2000
- "Economic impact of TB"
- **Dr.Rajeswari Ramachandran**
-"Social impact of TB"- **Dr.Rani Balasubramanian**

Meeting on Global Alliance for anti-TB drug development held at Geneva duirng Sept.2000
- **Dr.T.Santha Devi**

Meeting on Future coordination of clinical trials for tuberculosis and on future development of Rifapentine held at WHO, Geneva during Dec.2000
- **Dr.T.Santha Devi**

Advocacy

RNTCP Training for District TB Officers, conducted by Ministry of Health held at LRS Institute of TB and allied Diseases, New Delhi during July 1999
- **Dr.Rajeswari Ramachandran** (Facilitator)

Course on 'Training of the trainer' organised by DANTB, Orissa. held at Konark, Orissa during Aug.1999
- **Dr.Rani Balasubramanian** (National Trainer)

Temporary Advisor to Regional Director, WHO in 3rd regional training course on TB Control in South East Asia held at National TB Centre, Kathmandu during Sept.-Nov. 1999 - **Dr.Rajeswari Ramachandran**

Master's training in TB programme held at Indonesia during Nov.2000 - **Dr.AleyammaThomas** (invited by WHO as facilitator)

Contact Persons

Operational Research & Training

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Dr.K.C.Umapathy
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EPIDEMIOLOGY

OVERVIEW

This division has over the years built up considerable capability in conducting large scale epidemiological field trials and surveys. It has developed qualitative as well as quantitative methodologies for carrying out epidemiological studies in tuberculosis, These have been successfully adopted by other centers both nationally and internationally. This expertise has also been found useful for the study of other infectious diseases such as leprosy, lymphatic filariasis and typhoid in the same area. The strength of the department is the availability of a large number of staff who possess specialized skills requisite for field epidemiology and their proven ability to involve the communities in all aspects of the study. This unit was primarily responsible for the conduct of the classic BCG Chingleput study. The division is currently involved in a large study involving 500,000 population to assess the epidemiological impact of DOTS. In a consultative capacity the unit is assisting the Govt. of India in carrying out a national sample survey to estimate the burden of tuberculosis in the manner.

NEW INITIATIVE

Tuberculosis Prevalence / Incidence trend analysis (Infection & Disease)
Assessment of the epidemiological impact of "DOTS Strategy" of RNTCP

RESEARCH FOCUS

Tuberculosis Epidemiology

- **Disease Survey**
- **ARTI Survey**
- **Intervention strategies**
- **Assessment of risk factors**

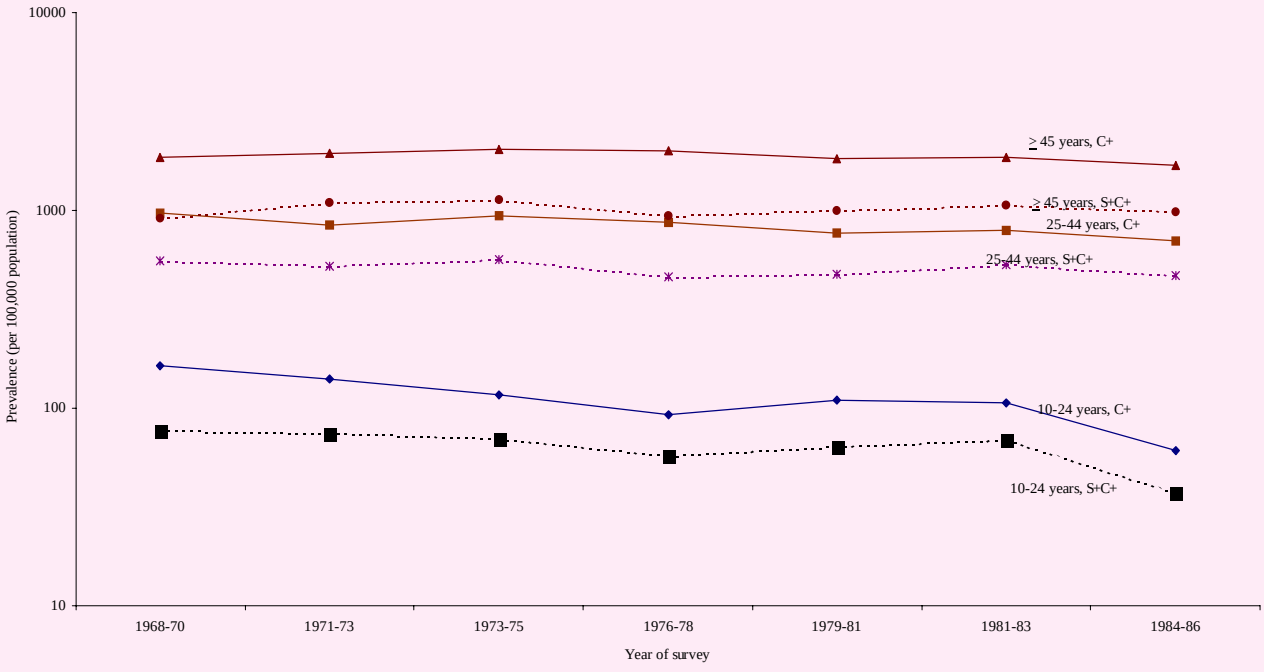
Trends in the prevalence and incidence of tuberculosis in south India

The natural history of tuberculosis is a dynamic process. Inspite of the availability of effective chemotherapeutic regimens for treatment, the deadly killer disease could not be effectively controlled to the level of preventing its transmission dynamics. The adoption of the "DOTS strategy" as an intervention measure by the Govt. of India on a national basis needed evaluation in terms of its impact on the epidemiology of the disease. As the assessment of any intervention strategy in a community requires the availability of pre and post intervention disease status, the study of the epidemiological trend of tuberculosis in the study area becomes mandatory. The available information from the Tuberculosis Prevention Trial data way back from 1968 was analyzed to assess the situation.

In 1968-70, a prevalence survey was undertaken on a population of about 100,000 persons in six blocks of Chingleput district in South India. All study subjects were followed for a period of 15 years by periodic re-survey (once every 2½ years), selective case finding and passive case detection, and new entrants were included at every re-survey. Radiographic examination of all subjects aged 5 years or more, and sputum smear and culture examination of those with an abnormal shadow were undertaken; tuberculin tests were done initially on all, and at 4, 10 and 15 years in selected samples of subjects aged 1-9 years. Rates were standardized by age and sex using the direct method.

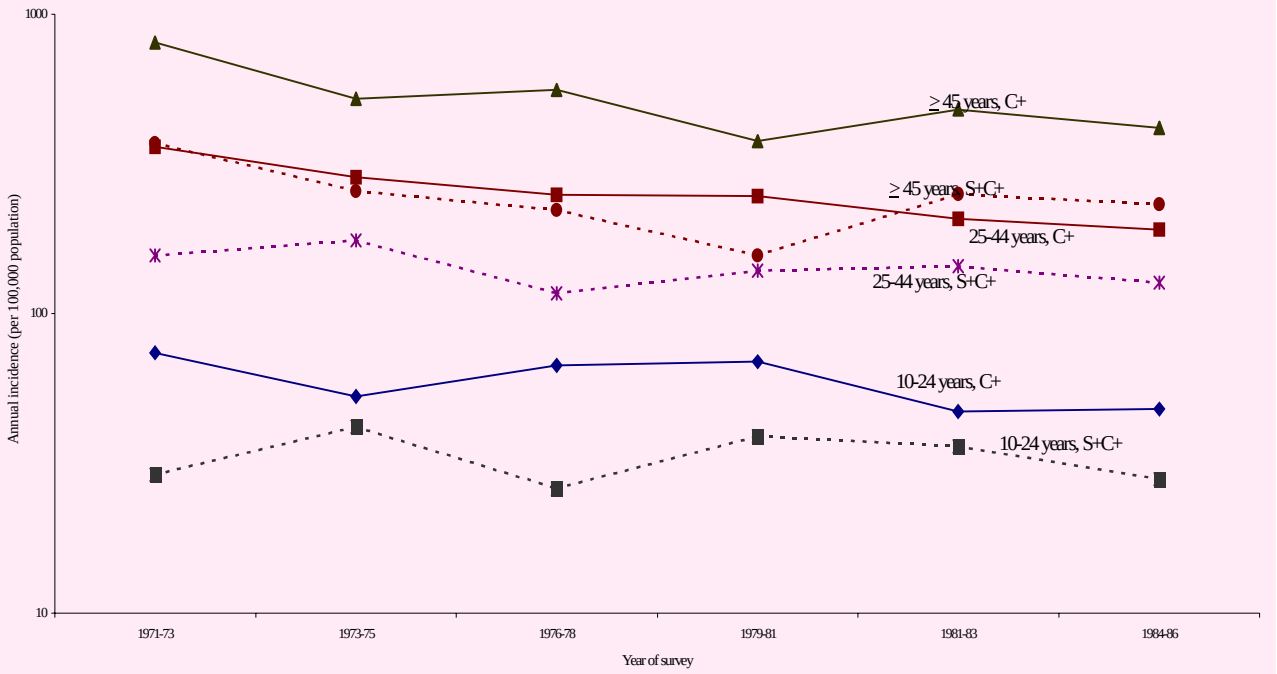
The prevalence of culture-positive tuberculosis decreased by 1.4% per annum over the entire study period (1968-86), from 875 to 694 per 100,000 and that of smear-positive, culture-positive tuberculosis showed no significant decrease, being 457 in 1968-70 and 428 per 100,000 in 1984-86 (Fig.1). The annual incidence of culture-positive tuberculosis was 352 per 100,000 at the first re-survey and it decreased subsequently by 4.3% per annum, while that of smear-positive, culture-positive tuberculosis

Fig.1 Age-specific trends in the prevalence of tuberculosis (both sexes combined)



was 157 per 100,000 and decreased by 2.3% per annum, to 113 per 100,000 (Fig.2). Decreases in incidence occurred exclusively in those who had abnormal radiographic findings suggestive of tuberculosis at the start of the period; among patients whose baseline chest radiograph was normal, the incidence did not decrease. It may be speculated that the substantial decrease in incidence observed was due to increased resistance to disease following improvement in socio-economic status, or to a greater likelihood of subjects with radiographic disease having received anti-tuberculosis treatment on account of increased availability of drugs.

Fig.2 Age-specific trends in the incidence of tuberculosis (both sexes combined)



The ratio of prevalence to annual incidence was about 3.5 for smear or culture-positive tuberculosis. The Annual Risk of Tuberculosis Infection (ARTI) is a useful epidemiological index for studying changes in the magnitude of the burden of tuberculosis in the community. It was 2.0% initially and showed no sign of decline over the 15-year period. For each 1% annual risk of infection, there were approximately 57 new smear-positive cases of tuberculosis per 100,000 population.

A subset of 2 blocks (Kadambathur & Thiruvalangadu) was studied over a longer period, including surveys in 1991-92 and 1994-96. The mean prevalence of culture-positive tuberculosis was 702 per 100,000 in 1968-70, declined at the rate of 1.2% per annum ($P<0.001$);and that for smear-positive tuberculosis was 398 in 1968-70, declined at the rate of 2.7% per annum ($P<0.001$).

The incidence of culture-positive tuberculosis was 356 per 100,000 per annum at the first re-survey (1971-73) and decreased subsequently at the rate of 1.5% per annum ($P>0.2$); the decrease was 2.9% per annum for smear-positive tuberculosis ($P<0.01$).

The epidemiology of tuberculosis in Chingleput district is known more than any other community in a high prevalence area. In 1998, a re-survey was begun to determine the prevalence, incidence and ARI in the area. In mid-1999, the WHO-recommended strategy of DOTS was implemented. Over the coming years, the effect of DOTS implementation on the prevalence and incidence of tuberculosis infection and disease will be documented.

National Sample Survey (annual risk of tuberculosis infection) in South India

The Tuberculosis Research Centre's commitment to the National Sample Survey – Annual Risk of Infection is twofold. Firstly, to train the health workers from all the four zones and secondly, to carry out the survey in south zone.

The training activity commenced on 11.10.1999 for the first batch of trainees from South Zone (Team – I). Subsequently, South Zone (Team – II), North Zone (Teams – I & II) and West Zone (Team – II) have completed their training. The last batch completed its training on 20.08.2000. Each team was trained for a period of two months in Census taking, Tuberculin Testing and Tuberculosis Reading.

The objective of the survey was to estimate the prevalence of tuberculosis infection and compute ARTI among children aged 1-9 years in South Zone. The South Zone comprises of Karnataka, Andhra Pradesh, Tamil Nadu and Kerala.

Six districts were randomly selected from South Zone. 600 clusters were distributed to these six districts proportionate to their population size. In each cluster, 85 children will be test/read. The survey was inaugurated on 05.01.2000 in Dakshin Kannada district of Karnataka. So far, Dakshin Kannada (Karnataka), Medak (Andhra Pradesh),Belgaum District (Karnataka) and Kanyakumari (Tamil Nadu) districts have been completed. The study is in progress.

Association between tobacco smoking and pulmonary tuberculosis: A nested case-control study

This study was conducted to evaluate the risk of developing tuberculosis among tobacco smokers. The study 'cases' and 'controls' were selected from the nested population of tuberculosis disease survey in random sample of villages selected from Kadambatur and Tiruvalangadu blocks of Tiruvallur district. A case is defined as "a male sputum positive (smear and culture) pulmonary tuberculosis patient detected in the survey between the age group 20-50 years" and a control is defined as "a male individual declared as a non-case in the survey between the age group 20-50 years" (Neighbourhood controls). The ratio between the case and control is 1:5.The information on tobacco smoking status, duration of smoking and the number of tobacco smoked per day were collected from the cases and the controls through a questionnaire. The outcome is that there is an association between tobacco smoking and pulmonary tuberculosis (Odds ratio = 2.5; 95% CI = 1.6 to 4.2) (Fig.1). The dose response curve shows a highly significant trend and the cumulative effect of duration (Fig.2) of smoking also shows a highly significant trend.

This study was presented in the international scientific meeting tobacco smoking and pulmonary tuberculosis sponsored by W.H.O and C.D.C Atlanta held on 17th and 18th November 2000 at Trivandrum in Kerala, India.

Fig. 1 Association between tobacco smoking and pulmonary tuberculosis

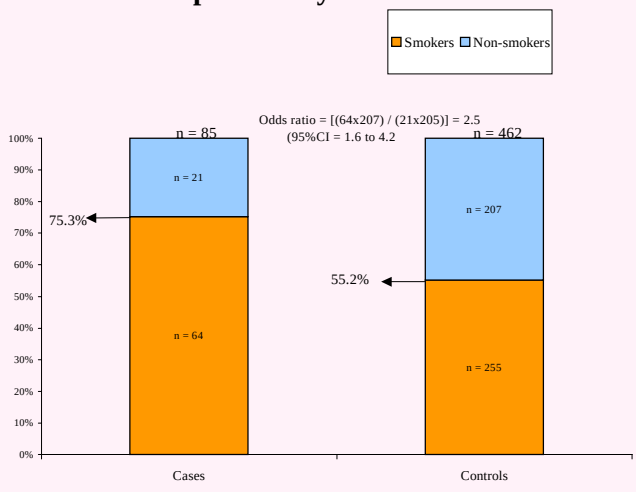
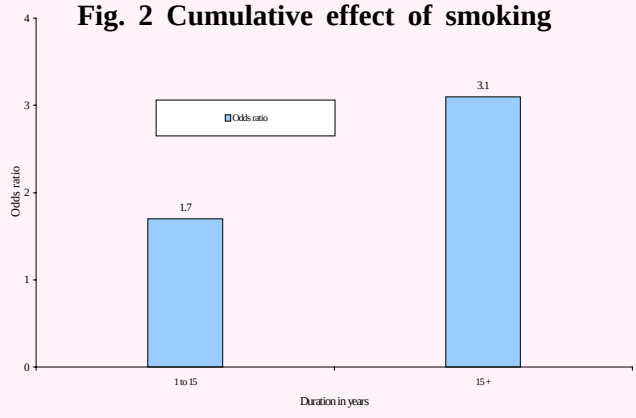


Fig. 2 Cumulative effect of smoking



Electronic Data Processing

Electronic data processing division is continuing data entry and verification and data management support to various studies undertaken in the Centre. The Centre has a total of 61 PCs, one mainframe VAX-11/750 computer and one local area network server which have been recently installed. This division is involved in retrieving, tracking and analysing vast collections of data present in different forms.

The following table gives the quantum of documents entered and verified in the year 1999 which has steadily increased in 2000.

Year	No. of documents entered	No. of documents verified
1999	1,78,198	1,79,730
2000	2,41,679	2,35,137

Computerization of recent studies such as Disease survey, ARI & Model DOTS project has been completed as seen in the accompanying table.

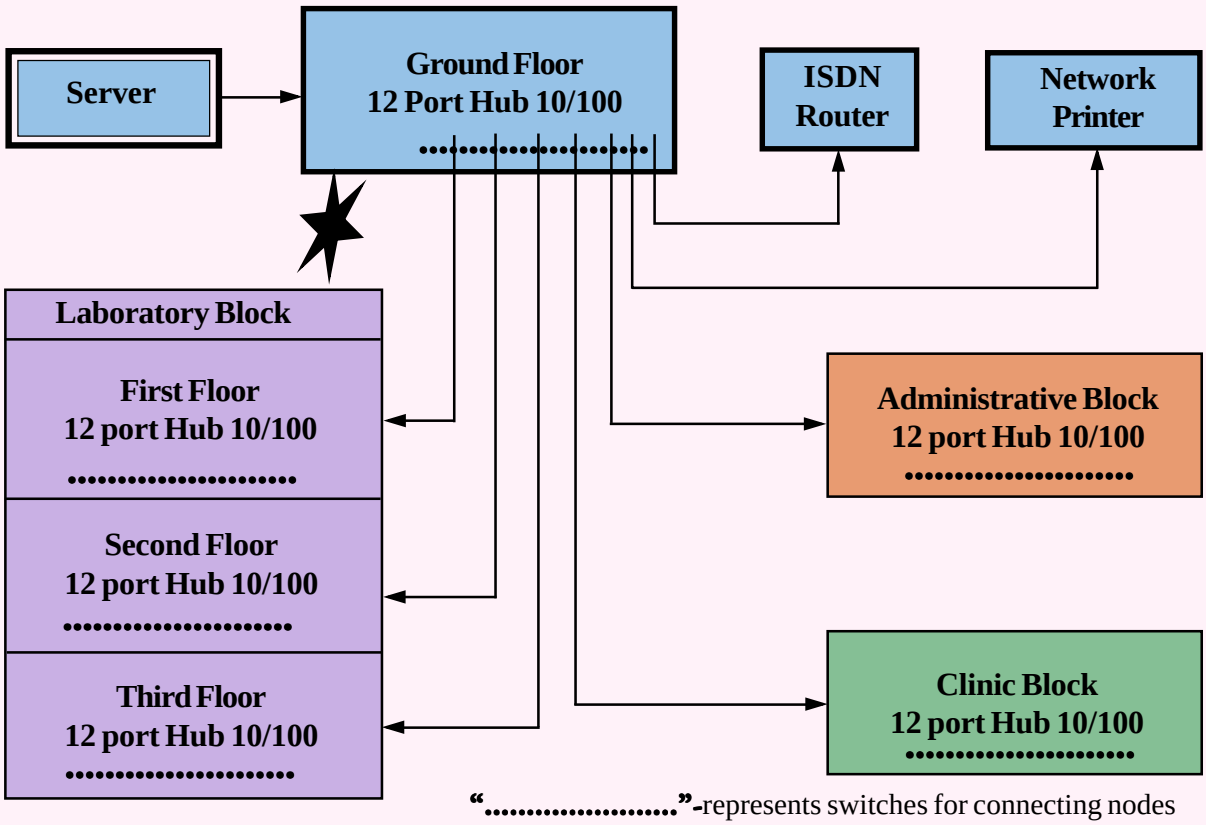
The data base files pertaining to these studies have been organised in the PC and routine data output is being made available to the Statistical Division for further action and monitoring purposes. Since the VAX-11/750 computer has become obsolete now, it has become absolutely necessary to create and store the date files in the PC.

Study	No. of records
1. Disease survey	79,000
2. ARI study	31,000
3. Model DOTS project	1,400
4. Clinic	1,368

Local Area Networking (LAN) of computers:

In order to make interaction/communication among scientists (nationally & internationally) more effective, efficient and easy, LAN facility has been created at TRC. It is now possible to have easy access to information network in a very short span of time. ISDN facility has been established to surf speedily and effectively.

Schematic diagram of LAN connectivity



"....."-represents switches for connecting nodes

Staff list

C.Kolappan, M.B.B.S., M.Sc.(Epid)
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P.G.Gopi, M.Sc.
R.Selvaraj, M.Sc.
R.Subramani, M.Sc.
P.Paul Kumaran, M.B.B.S.
R.S.Nagabushana Rao, B.Com.
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N.Shivaramu, 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.
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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
M.Venkatarao, B.Sc., D.M.L.T.
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.
A.Prasad Rao
P.Narayanan
G.K.Loganathan, M.A.
R.Krishnamurthy, B.A.
S.C.Ramaiah
H.Dhanasekaran
K.V.Parandaman, B.A.
K.Somasekar
S.Dakshinamurthy
S.Kumar, B.Com.

Capacity Building

Dr.P.Paul Kumaran - IUATLD course on TB Epidemiology-"Epidemiological basis of Tuberculosis Control – Hans L. Reader" held at National Tuberculosis Institute, Bangalore - June 1999

Mr.K.Balasubramaniam, Mr.E.Sakthivelu - Training course on Oracle Developer and Visual Basic-6 conducted by the Stenographer Guild, Chennai - Sept.1999

Dr.K.Sadacharam - Post Graduate Diploma in Computer Application at SISI Academy (Govt. of India) Oct.1999 – Nov. 2000

Dr.K.Sadacharam - Epidemiological basis of Tuberculosis Control at National Tuberculosis Institute, Bangalore (Training Course) - Jan.2000

Participation in Conferences / Workshops / Symposia

WHO / RNTCP training for Medical Consultants held at New Delhi during Aug.1999 - **Dr.P.Paul Kumaran**

Workshop on 'Sample size consideration in medical research' organised by the Dept. of Preventive and Social Medicine, Govt. Medical College, Nagpur and Maharashtra Chapter of IAPSM held at Nagpur during Aug.1999 - **Mr.P.G.Gopi**

WHO/ICMR Workshop on Health Research Management held at Chennai during Sept.1999
- **Dr.P.Paul Kumaran**

XVII ISMS conference held at National Institute of Mental Health & Neuro Science, Bangalore during

Dec.1999 - "Estimating of J-Shaped risk response relationship on Tuberculous Infection using Neural Networks" - **Mr.R.Selvaraj**

WHO Training for RNTCP Medical Consultants on EPI-INFO, Survey Design and Analysis held at Chennai during Dec.1999 - **Dr.P.Paul Kumaran**

5th International Conference on Emerging Infectious Diseases in the Pacific Rim held at Chennai during Jan.2000 - **Dr.P.Paul Kumaran**

Symposium on 'Status of Tuberculosis at 2000' organised by the Sir Dorabji Tata Centre for Research in Tropical Diseases, Dept. of Microbiology and Cell Biology, Indian Institute of Science held at Bangalore during Mar.,2000 - "Status of BCG vaccine" - **Dr.P.Paul Kumaran**

TB: an overview. Scenario during the Millennium 2000 - Commemorative publication on TB on 24th Mar.2000 (World TB Day) by Karnataka State TB Association and Action AID - India, Bangalore - **Dr.P.Paul Kumaran**

Review meeting on the ongoing efforts of assessment of TB incidence/prevalence in the country under the chairmanship of Director General of Health Services held at Nirman Bhavan, New Delhi during Apr.2000 - **Dr.C.Kolappan**

Experts group meeting held at National Tuberculosis Institute, Bangalore during June 2000 -**Dr.C.Kolappan**

Meeting on Tobacco smoking and pulmonary tuberculosis organised by Tata Institute of Fundamental Research and sponsored by World Health Organisation and C.D.C., Atlanta held at Trivandrum during Nov.2000 - **Dr.C.Kolappan**

Advocacy

The Epidemiology unit has participated in the Chief Minister's Health Camp in Tiruvallur District and in Jawadhu Hills among Tribals Population in Tamil Nadu.

Contact Persons

Disease Survey
Annual Risk of Tuberculosis Infection
Field

Dr.C.Kolappan
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Smoking and Tuberculosis

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Statistics

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Molecular Epidemiology

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Tuberculosis Key Facts

❖ More adults die from TB than from any other infectious disease- 1 every minute, more than 1,000 every day in India. The National Tuberculosis Programme was begun in 1962 and created an infrastructure for TB control throughout the country. However, it has not achieved the desired results.

❖ Dr.Hiroshi Nakajima, former Secretary-General of the World Health Organisation, declared that "the DOTS strategy represents the most important public health breakthrough of the decade.

❖ The strategy of the Directly Observed Treatment, Short-course (DOTS) is based largely on research done in India in the field of TB over the past 35 years.

❖ Since 1993, DOTS has been pilot tested in 20 sites of India as the Revised National Tuberculosis Control Programme(RNTCP). In the RNTCP, the proportion of TB cases which are confirmed in the laboratory is double that of the previous programmes, and the cure rate is nearly triple that of the previous programme.

❖ The operational feasibility of DOTS in the Indian context has been demonstrated, with 8 out of 10 patients treated in the programme being cured, as compared with approximately 3 out of 10 in the previous programme.

❖ Multidrug-resistant tuberculosis(MDRTB) is a result and symptom of poor programme performance. Reliable and representative data on the rate of MDRTB in India is not available. DOTS has been shown to prevent the emergence of MDRTB and to reverse the trend of MDRTB in communities in which it has emerged.

❖ The Human immunodefiency virus (HIV) is the strongest known risk factor for development of TB. In some countries, HIV has tripled TB caseloads. However, DOTS can cure TB even in HIV-positive people.

❖ Success of the RNTCP depends on communication, collaboration and co-ordination between the Government and private practioners, non-governmental organisations and other institutions of prominence such as medical colleges.

❖ In the next three years, the RNTCP is to be implemented in a phased manner in a population of more than 300 million throughout India, and at the same time the rest of the country will be prepared for RNTCP implementation. Phased implementation is essential for success.

❖ By the year 2000, the number of infectious patients cured per year will increase from the current level of atmost 1,50,000 to more than 5,00,000 per year. By the year 2000, 1,00,000 fewer patients will die every year from TB as a result of the RNTCP. Every patient who is cured, stops spreading TB and every life saved is a child, mother, or father who will go on to live a longer, TB-free life.

Central TB Division, DGHS,
Ministry of Health & Family Welfare,
Government of India, New Delhi.

HIV/AIDS

OVERVIEW

The department of HIV/AIDS was created in February 2000 with a view to translate the commitment of TRC towards an increase in the quantum of research activity in the field of HIV/AIDS. The department is composed of a multi-disciplinary team of scientific and technical staff who will undertake both clinical and laboratory studies in the field of HIV with special emphasis on the interaction between HIV and tuberculosis. The department is currently conducting controlled clinical trials for the treatment and prevention of tuberculosis in HIV infected individuals, in collaboration with various state Government hospitals. In addition, studies have been initiated to understand the complex interplay between HIV and TB on the cell – mediated immune response and also to study the role of immune activation markers in assessing response to treatment and prognosis in patients with HIV and tuberculosis. Future plans include studies of opportunistic infection in HIV, pediatric HIV and the interaction between host genetics and the specific immune response.

NEW INITIATIVE

Establishment of Clinical Research Units at
i) Government Hospital for Thoracic Medicine, Tambaram
ii) Government General Hospital, Chennai

RESEARCH FOCUS

Chemotherapy of tuberculosis in HIV infected persons
Immune status, viral load and cytokine profile in patients with HIV/TB
Residual lung function impairment in pulmonary tuberculosis patients
Quality of life and the sexual and psychological health of patients with HIV/TB

Chemotherapy of pulmonary tuberculosis in HIV infected patients – An evaluation of intermittent Short Course (RNTCP) Regimens

Tuberculosis is the commonest infection occurring in HIV infected patients in India and studies from different parts of the country have estimated that 60 to 70% of HIV positive patients will develop tuberculosis in their lifetime. Tuberculosis occurs at different stages of the HIV disease and its presentation and response to treatment also differ accordingly. Tuberculosis occurring during the early asymptomatic phase of HIV infection behaves in a manner similar to that in immunocompetent individuals. However, the clinical presentation and response to treatment in patients with immunodeficiency (CD4 counts < 200/cu.mm) is often atypical. There have been a few recent reports stating that intermittent short course regimens are effective in HIV infected patients with TB; however some of these studies have indicated higher recurrence rates compared to tuberculosis patients without HIV.

In India, the Revised National Tuberculosis Control Program (RNTCP) is being introduced in a phased manner all over the country and envisages that all tuberculosis patients will be treated with intermittent short-course regimens using the DOTS Strategy. The response of patients with HIV and TB to these particular regimens has not been studied in India.

The objectives of the present study are:

i. To evaluate the efficacy of RNTCP (intermittent short course) regimens in patients with HIV and tuberculosis and to investigate an alternative Category I regimen with a longer continuation phase.

ii. To study the relationship between stage of HIV disease and response to anti-TB treatment.

iii. To study the relapses and their nature in detail by using RFLP analysis

Methodology

Patients attending the clinic at Tuberculosis Research Center at Chennai and Madurai who are known or suspected to be HIV positive and also have chest symptoms are admitted to the study if they fulfill eligibility criteria. Patients are investigated and categorized to receive one of three regimens based on RNTCP guidelines. It is proposed to admit 500 patients to this study. Patients will receive one of the following regimens:

Category I (New sputum smear positive pulmonary TB): **$2EHRZ_3/4RH_3$** versus **$2EHRZ_3/7RH_3$**. Patients will be randomly allocated to either of these regimens, after stratification based on CD4 counts.

Definition: TB in a patient with at least 2 initial sputum smear examinations positive for AFB or 1 sputum positive for AFB and radiographic abnormalities consistent with active pulmonary TB or 1 sputum specimen positive for AFB and culture positive for *M.tuberculosis*. Patient should have had no treatment for TB or should have taken anti-TB drugs for less than 1 month any time in the past.

Category II (Sputum smear positive pulmonary TB cases who are relapses, treatment failures or defaulters): **$2SEHRZ_3/1EHRZ_3/5EHR_3$**

Definition: Smear positive pulmonary TB as defined above

Relapse: A patient declared cured of TB but who reports back to the health service and is found to be bacteriologically positive.

Treatment failure: A smear positive patient who is smear positive at 5 months or more after starting treatment or a patient initially smear negative who becomes smear positive during treatment.

Treatment after default: A patient who received anti-TB treatment for one month or more from any source and who returns to treatment after having defaulted i.e not taking anti-TB drugs consecutively for two months or more.

Category III (Sputum smear negative pulmonary TB, extrapulmonary tuberculosis, not seriously ill): **$2HRZ_3/4RH_3$**

Definition: A patient with symptoms suggestive of TB with 6 initial sputum examinations negative for AFB and radiographic abnormalities. Such patients will be given a 10 day course of broad spectrum antibiotics (Bactrim DS 2 bd) and Chest X-ray repeated after 15 days. If the radiographic abnormality persists, then the decision to treat as TB will be made by a panel of doctors. 4 sputa will be repeated at this point. Patients with extra-pulmonary tuberculosis like TB lymphadenitis, pleural effusion etc who are not seriously ill will be treated with this regimen

All anti-TB drugs will be administered as per the RNTCP strategy of DOTS. Patients residing in Chennai

and Madurai will attend the respective centers/ subcentres three times a week for the intensive phase of treatment (first two/three months) and then once a week during the continuation phase (four to seven months). For patients from outside our area of intake, DOTS providers will be identified from the community and supervised by TRC field staff.

Preliminary Results

Up to October 31, 2000, 317 patients had been registered and 87 patients (76 male, 11 female) admitted to the study. The age range was from 20 to 60 years with a median of 32 years. 67 patients had completed treatment during this period, of whom 41 were excluded from the analysis for various reasons (sputum culture negative 11, HIV negative 6, defaulter for > 1 month during the treatment period 18, and death within 1 month 6). Of the 26 patients in analysis, 21 patients had been admitted to Category I, 2 to Category II and 3 to Category III.

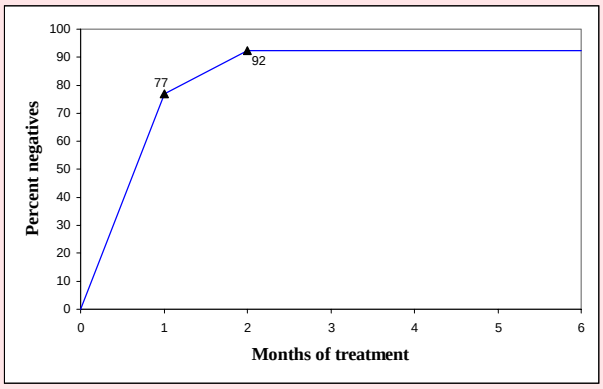
The proportion of patients becoming culture negative at different months and the drug susceptibility profile of pre-treatment cultures for 54 patients are shown in the following figure & table respectively.

Drug susceptibility profile

Sensitive to all drugs	46
Resistant to Isoniazid (H)	6
Resistant to Rifampicin (R)	1
Resistant to Streptomycin (S)	2
Resistant to HR	1
Resistant to S,H,R,E	1
Total	54

43 patients (80%) had organisms sensitive to all first line drugs.

Culture conversion during treatment period



24 out of 26 patients (92%) had a favourable response at the end of treatment. There were 5 relapses (20%), all occurring within 6 months of stopping treatment. There were 6 deaths in the follow-up phase (4 of these were due to causes other than TB and 2 due to TB).

Expected Outcome

The study will provide information on the response of HIV infected tuberculosis patients to the anti-TB regimens currently recommended in the Revised National TB Control program. In addition, it will allow us to study recurrent TB in this population and the relative contribution of re-infection and endogenous re-activation.

Residual lung function impairment in patients treated for pulmonary tuberculosis

The objective of this pilot study was to determine the prevalence of pulmonary function abnormalities among patients who had been treated for pulmonary tuberculosis with standard short course regimens. Twenty-three patients who had completed treatment within the past 24 months and continued to be sputum smear and culture negative for *M.tuberculosis* were studied. They were assessed with pulmonary function tests and room air blood gas analysis. The results are shown in the following table:

Pulmonary function test results

Parameters* (n = 23)	No. of patients predicted		
	≥ 80%	79- 60%	≤ 59%
Forced Vital Capacity (FVC)	10	9	4
Forced Expiratory Volume in 1 sec (FEV1)	11	9	3
Maximal Voluntary ventilation (MVV)	11	7	5
Peak Expiratory flow Rate (PEFR)	7	9	7
Forced Mid Flow (FMF)	6	5	12
Flow at 25% of FVC (VE25)	10	5	8
Flow at 50% of FVC (VE 50)	10	5	8
Flow at 75% of FVC (VE 75)	10	2	11

*Patients with values less than 80% predicted are considered to have moderate to severe disease

56% of these patients had spirometric test abnormalities with reduction in Forced Vital Capacity (FVC) and Maximal Voluntary Ventilation (MVV). 70% of the patients had maximal expiratory air flow limitation suggesting airway obstruction. 48% had abnormally high residual volume indicating air trapping. 74% had Carbon monoxide diffusion impairment and 56% had arterial blood hypoxemia (PaO_2<90mm Hg) at rest suggesting diffusion abnormalities. These patients also had high alveolar-arterial oxygen gradient and increased dead space ventilation, indicating ventilation perfusion abnormalities. On further analysis it was observed that arterial hypoxemia correlated with decrease in flow rates.

The results from this pilot study suggest that patients successfully treated for pulmonary TB have significant residual lung function impairment. The pulmonary function defect is of mixed type with airway obstruction predominantly, leading to hypoxemia in half of the cases. It is proposed to extend and continue this study to include at least 100 patients and to study them more extensively with exercise stress testing as well.

Cytokine response in HIV patients co-infected with *M.tuberculosis*

Active tuberculosis is now recognized as a frequent and serious complication of infection with the human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS) in man.

Protective immunity against *M.tuberculosis* is based on cell-mediated immunity, involving bi-directional interactions between T cells and cells of the monocyte-macrophage lineage. Some of the key factors involved in this interaction include T-cell derived interleukin-2 (IL-2) and interferon-gamma (IFN-γ), as well as monocyte-derived IL-12 and tumor necrosis factor-alpha (TNF-α). Quantitative and qualitative defects in T lymphocyte function that result from direct HIV infection of CD4+ cells, severely limit the production of macrophage-activating cytokines capable of inducing an anti-mycobacterial state in cells of the monocyte lineage in HIV patients, leading to more extensive and life-threatening disease in HIV patients than in immunocompetent individuals.

Co-infection of HIV patients with *M. tuberculosis* also contributes to the progression of HIV disease, but so far, the mechanisms involved are not clear. Data suggest that the microenvironment generated by tuberculosis might up regulate HIV replication, partly through cytokines like TNF-α and IL-6.

Anti-tuberculous therapy is known to improve the immunological status of HIV positive tuberculosis cases. Studies have shown a slight increase in lymphocyte proliferation and IFN-γ production, and a marked increase in the CD4/CD8 ratio and cytotoxicity following treatment.

Objectives

To investigate specific alterations in the immune response in HIV-positive TB patients brought about by anti-tuberculous therapy(ATT) by studying the following immune parameters before (day 0), during (2 months) and after (6 months) ATT:

i. IFN-γ, TNF-α, IL-6, IL-8, IL-12 and IL-18 production in cell cultures stimulated with mycobacterial antigens *in vitro*

ii. IL-2 receptor (IL-2R), IL-8 receptors (IL-8RA/CXCR-1 and IL-8RB/CXCR-2) and fibroblast-associated apoptosis-1 soluble receptor (Fas/sApo-1) expression in response to mycobacterial antigens

iii. CD4 and CD8 levels

iv. Viral burden

Methodology

Study subjects: The study group will comprise of 20 individuals dually infected with HIV and *M.tuberculosis*, 20 patients with HIV alone, 20 with TB alone as well as 20 normal volunteers.

Antigens and mitogens: Purified protein derivative (PPD, 10 µg/ml) and heat-killed *M.tuberculosis* H37Rv (Hk-Mtb, 10 µg/ml) will be used as parasite antigens for *in vitro* stimulation of cell cultures, while phorbol myristoyl acetate (PMA, 50 ng/ml) along with ionomycin (1 µg/ml) will serve as the mitogenic stimulus.

Isolation and culture of lymphocytes: 20 ml of venous blood will be collected in EDTA and subjected to Ficoll-Hypaque density gradient centrifugation. The mononuclear cells will be isolated and their viability assessed by trypan blue dye exclusion method Lymphocytes will be cultured at a concentration of 2 x 10^6 cells per well in 1 ml of complete RPMI-1640 medium containing 10% fetal calf serum in the presence of PPD, Hk-Mtb, PMA+ionomycin, or medium alone, at 37^0C. Culture supernatants will be collected after 24 and 72 hours of incubation and stored at -70^0C until the time of assay.

ELISA: Capture ELISA will be utilized to quantitate the levels of the cytokines and activation markers mentioned above in the stored culture supernatants as per the standard protocol.

Flow cytometry: 75µl of whole blood will be stained with the following combinations of monoclonal antibodies: CD3 FITC/CD19 PE, CD3 FITC/CD4 PE, CD3 FITC/CD8 PE & CD3 FITC/CD16+CD56 PE and analyzed using a FACSort flowcytometer (Becton Dickinson). The SimulTest Leucogate (CD45 FITC/CD14 PE)will be used to identify the lymphocyte population. The number of cells belonging to each subtype will be determined by multiplying the percentage of those cells by the absolute lymphocyte count.

Viral load assay: RNA will be extracted from plasma samples of all HIV positive cases and the viral burden determined using the fully automated COBAS AMPLICOR (Roche) following the manufacturer's protocol.

Differences in cytokine production between groups will be studied, and their levels will be correlated with viral load and CD4/CD8 counts in HIV positive patients.

Health related quality of life in patients with HIV/TB

Patients with HIV/TB have a variety of health related problems which affects 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 at the end of treatment.

Patients are specifically asked about their health and emotional problems in an easily understandable way. The questionnaire used is a self report one that has been developed by Gordon H Guyatt. The items are designed to study the degree to which a patient feels control over the disease, its manifestations and emotional dysfunctions. A visual analog scale is used to grade responses.

Fifteen patients with HIV/TB have completed the questionnaire before start of anti-TB treatment. All these patients will be questioned at the completion of their treatment. It is planned to include 50 patients in this study, before their anti-TB treatment is started. All these patients will be questioned at the completion of their treatment.

Assessment of the sexual and psychological health of HIV positive and HIV negative women in India

This is a cross sectional pilot study aimed at providing a socio culturally based assessment of sexual behavior, contraceptive use, relationship factors and psychological distress in a sample of 300 HIV positive and 300 HIV negative women in two regions of India. (Chennai and Delhi). Specifically, it will examine demographic and socio cultural factors influencing sexual decision making and high risk behavior and examine the differences between the two groups of women.

The long term goal is that both assessment and intervention programmes as well as staff training be incorporated into various medical curricula in order to maximize efforts to reduce the rates of women's infection with HIV / AIDS.

The two sites in Chennai involved in this study are the Tuberculosis Research Centre and Govt. Hospital for Thoracic Medicine, Tambaram.An initial training workshop was conducted for the personnel involved in the project from Delhi and Chennai from Oct 16th – 20th at TRC, Chennai. This a collaborative study between ICMR and UCLA.

The project is time targeted and is expected to be completed by March 2001.

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.
N.Arun Kumar, M.Sc.
Annamma Joy
Mary Unice George

Awards

Dr. Keya Lahiri Gold medal for best paper presented at XI National Pediatric Pulmonary Conference held at Calcutta during Sept.1999 -"A profile of culture confirmed pulmonary tuberculosis in children"
- Dr.Soumya Swaminathan

Capacity Building

Attended a course on "International Epidemiology of HIV/AIDS" at the School of Public Health, University of California, Los Angeles, USA during Apr.-June 2000. Also attended a series of lectures in HIV Immunology and learnt techniques to asses cell- mediated immune responses at the UCLA AIDS Institute, California (Dept. of Microbiology and Immunology) - **Dr.Soumya Swaminathan**

Thesis in progress

Mrs.Luke Elizabeth Hanna -Compartmentalization of immune responses - The Tamil Nadu Dr.MGR Medical University.

Training in 'Cellular Immunology' held at National AIDS Research Institute, Pune during Nov.-Dec.2000
- Dr.Sudha Subramanyam, Mrs.Luke Elizabeth Hanna, and Mr.K.Sankaran

Participation in Conferences / Workshops / Symposia

National

ICMR-WHO National Workshop on 'Health Research Management' held at Chennai during Sept.1999
– **Dr.Soumya Swaminathan**

20th Annual Conference Indian of the Association of Biomedical scientists held at Chennai during Oct.1999 - "Alveolar – Arterial oxygen gradient in patients treated for pulmonary tuberculosis"
- **Dr.K.V.Kuppu Rao**

National Conference on Pulmonary Diseases NAPCON held at New Delhi during Nov.1999 - "Reduced Exercise Capacity in children with bronchiectasis" - **Dr.Soumya Swaminathan**
-"Residual lung function impairment in patients treated for pulmonary tuberculosis" - **Dr.K.V.Kuppu Rao**

Sub-Regional Seminar on "Innovation in Early childhood care and development" organised by UNICEF, held at Chennai during Nov.1999
- **Dr.Soumya Swaminathan**

37th National Conference of the Indian Academy of Pediatrics held at Hyderabad during Jan.2000 - " Multi drug resistant TB " - **Dr.Soumya Swaminathan**

IAP Respiratory chapter, Training program in Pediatric pulmonology held at Chennai during Mar.2000
- " Introduction to PFT's and Multidrug resistant TB "
- Dr.Soumya Swaminathan

Asian Regional Training Workshop on HIV/AIDS Vaccine Research Ethics held at New Delhi during June 2000 - **Dr.Soumya Swaminathan**

Indian Academy of Pediatrics Core group meeting of TB chapter held at New Delhi during Aug.2000 -
" Diagnosis of Childhood TB "
- Dr.Soumya Swaminathan

National Academy of Medical Sciences sponsored guest lecture held at Child's Trust Hospital, Chennai during Aug.2000 -"Ethical Issues in HIV Research"
- Dr.Soumya Swaminathan

American College of Chest Physician Chest Meet 2000, held at Chennai during Aug.2000 -"Pros and Cons of DOTS" - **Dr.Soumya Swaminathan**

Dr. C.W. Chacko Memorial Oration, Indian Association for the study of Sexually Transmitted Diseases and AIDS(IASSTD and AIDS) held at Kilpauk Medical College, Chennai during Sept.2000 - "Recent Advances in HIV – Perinatal infection, prevention and management " - **Dr.Soumya Swaminathan**

Biothirst Symposium, Alpha College held at Chennai during Sept.2000 -"The Threat of Infectious Diseases"
- **Dr.Soumya Swaminathan**

XI National Pediatric Pulmonology Conference held at Tirupati during Oct.2000 - "Pulmonary Function Tests"
- **Dr.Soumya Swaminathan**

III National Conference on Pediatric Infectious Diseases held at Chennai during Nov.2000 - "Perinatal HIV Infection" - **Dr.Soumya Swaminathan**

Membership in Expert Committees & Special Assignments

National Editorial Advisory Board of the Indian Journal of Pediatrics, New Delhi - **Dr.Soumya Swaminathan**

Editorial Committee of the Indian Journal of Practical Pediatrics, Chennai - **Dr.Soumya Swaminathan**

Guest Editor, Mini Symposium on Pediatric TB, Pediatric Respiratory Reviews, London , U.K
- Dr.Soumya Swaminathan

Member, TB Working Group, Indian Academy of Pediatrics - **Dr.Soumya Swaminathan**

Guest Editor, Special Supplement on Tuberculosis, Indian Journal of Pediatrics, Jan.2000
- Dr.Soumya Swaminathan

Member of Organizing committee CHEST MEET 2000 organized by American College of Chest Physicians, South India Chapter held at Chennai during Aug.2000
- **Dr.K.V.Kuppu Rao**

Training Programmes

Medical students, interns and postgraduate students from various medical colleges in and around Chennai underwent training in principles and application of pulmonary function testing as well as in topics related to HIV and tuberculosis.

Advocacy

Indo-US (ICMR–UCLA) training program for investigators in the "Study of sexual and psychological health of HIV positive and negative women in India" held at Chennai during Oct.2000 - **Dr.Soumya Swaminathan**

National Protocol Development Workshop on "HIV and tuberculosis" held at Chennai during Nov.2000
- Dr.Soumya Swaminathan

Contact Persons

Clinical and laboratory aspects of HIV infection
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Pulmonary function tests
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BACTERIOLOGY

OVERVIEW

This Department is one of the largest facilities of its nature in South East Asia and also serves as a WHO Reference Laboratory. The laboratory routinely provides support for all controlled clinical trials, programme-based studies and evaluates drug resistance for referral samples. It also supplies standard strains and clinical isolates of mycobacteria to other investigators. The laboratory is studying the early bactericidal activity of anti- tuberculosis drugs and the molecular basis of drug resistance. Animal studies are being carried out to determine factors that influence virulence, dormancy and drug resistance. The department is also actively involved in development of early diagnosis of drug resistance. A team of well trained scientific and technical staff are involved in conducting several basic studies.

NEW INITIATIVE

Drug resistance surveillance
Evaluation of diagnostic test

RESEARCH FOCUS

Drug resistance surveillance
Mycolic acid analysis
Diagnosis of tuberculosis
Luciferase reporter phage
Molecular mechanisms of rifampicin resistance
Environmental mycobacteria
Dormancy in tuberculosis

Surveillance of drug resistance in two Districts in South India

One of the important recommendations of the Expert Group Meeting on Drug Resistance Surveillance in TB organized by the Central TB Division at this Centre in September 1997 was that a systematic ongoing surveillance on drug resistance in new TB patients should be undertaken on a continuous basis in order to provide information on programme performance. It was decided that TRC would undertake these 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 re-survey would enable an understanding of the trend in resistance over the years. The studies in North Arcot and Raichur were completed during the period 1999-2000.

The two districts of North Arcot and Raichur have since been bifurcated into two smaller districts each. In order to have a representative sampling, the study was undertaken in all the four districts, viz. Vellore and Tiruvannamalai (referred to as composite North Arcot district) as well as Raichur and Koppal (referred to as composite Raichur district), the results of which are reported here.

A total of 635 specimens from 320 patients were collected from North Arcot District during February-April 1999. In the Raichur district, 617 specimens were collected from 314 patients during July-December 1999.

The bacteriological investigations at TRC included smear examination, culture, drug susceptibility tests to streptomycin (S), isoniazid (H), rifampicin (R) and ethambutol (E). The isolates from patients in North Arcot district were also tested for susceptibility to ofloxacin (O). All isolates were subjected to identification tests for confirmation as *M.tuberculosis*.

On examination at TRC, 4.7% of 635 specimens from North Arcot and 5.7% of 617 specimens from Raichur district were found to be smear negative. Concordance in duplicate results from the same patient (within ± 1 grade) was observed in 92.5% of North Arcot patients and in 92.4% of Raichur patients(Table-1)

Table-1

Smear Grade	N.Arcot No.	N.Arcot %	Raichur No.	Raichur %
Neg.	30	4.7	35	5.7
1+	520	81.9	457	74.1
2+/3+	85	13.4	125	20.3
Total	635		617	

The proportion of culture negative specimens was 5.7% in North Arcot and 6.3% in Raichur district. The yield of 2+/3+ grades was obtained from nearly 2/3 of the specimens from both the districts. An excellent agreement in duplicate culture results from the same patient was observed in both districts. The number of patients with both cultures negative was 11 (3.5%) in North Arcot and 14 (4.5%) in Raichur. The proportion of patients lost from the analysis due to contamination of both cultures was identical, viz., 1.9% in each district(Table-2).

Table-2

Culture Grade	N.Arcot No.	N.Arcot %	Raichur No.	Raichur %
Neg.	36	5.7	39	6.3
⩽1+	105	16.5	143	23.2
2+	281	44.2	165	26.7
3+	166	26.1	245	39.7
Cont.	47	7.4	25	4.0
Total	635		617	

Drug susceptibility tests

a**) North Arcot district:** Out of 320 patients studied, 20 had yielded negative or contaminated cultures. Drug susceptibility tests were not available for 2 patients. Excluding these, there remained 298 patients, 282 with no history of previous treatment and 16 with previous anti-TB treatment(Table-3). Out of 282 patients with no history of previous treatment, 204 (72.3%) were found to be fully sensitive and 78 (27.7%) were resistant to one or more drugs. Any resistance to H was observed in 66 (23.4%) and any resistance to R in 8 (2.8%). All the latter patients were also resistant to H. The incidence of MDR-TB was 2.8% in this study. Of 16 patients with history of previous treatment as many as 13 (81%) were resistant to H. Eleven of these 13 patients were also resistant to R, the incidence of MDR-TB being 69%. No resistance to O was observed in both categories of patients.

b) Raichur district: Out of 314 patients, 21 had no culture result due to negativity or contamination. DS results were not available for 4 patients. Thus, out of 289 patients with results, 278 with no history of previous treatment and 11 patients with previous treatment remained in the analyses(Table-4). Of 278 patients in the former category, 217 (78.1%) were fully susceptible to all drugs and 61 (21.9%) had shown resistance to one or more drugs. Resistance to H alone or with other drugs, was encountered in 52 (18.7%) patients. Any resistance to R was seen in 7 (2.5%) patients. All these 7 were also resistant to H (MDR-TB) . All the 11(100%) patients with history of previous treatment were resistant to H and also to R (MDR-TB).

Table-3

	No History of Prev.Rx. Primary Resistance		History of Prev.Rx. Acquired Resistance	
	No.	%	No.	%
Totally Tested	282		16	
Fully Sensitive	204	72.3	3	18.8
Any Resistance	78	27.7	13	81.2
Any H Resistance	66	23.4	13	81.2
Any R Resistance	8	2.8	11	68.8
Any HR Resistance	8	2.8	11	68.8

Table-4

	No History of Prev.Rx Primary Resistance		History of Prev.Rx. Acquired Resistance	
	No.	%	No.	%
Totally Tested	278		11	
Fully Sensitive	217	78.1	0	0.0
Any Resistance	61	21.9	11	100.0
Any H Resistance	52	18.7	11	100.0
Any R Resistance	7	2.5	11	100.0
Any HR Resistance	7	2.5	11	100.0

The incidence of MDR-TB was 2.8% and 2.5% in North Arcot and Raichur districts respectively.

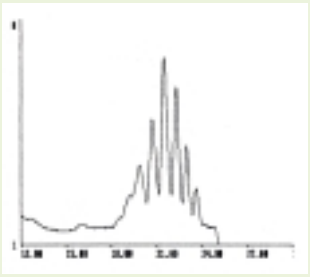
Speciation of mycobacteria by mycolic acid analysis

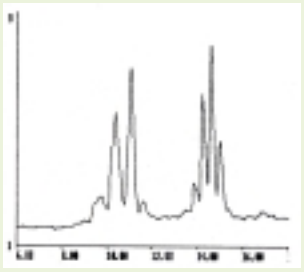
The possible increase in the incidence of opportunistic infections caused by nontuberculous mycobacteria (NTM) in patients with HIV brought a focus on isolation and identification of these species. Speciation is essential for the appropriate treatment, since NTM are innately resistant to most of the currently available antituberculosis drugs. Identification of NTM by biochemical methods is laborious and time consuming, whereas analysis of mycolic acid present in the bacteria by HPLC offers to be an accurate, rapid and economical method.

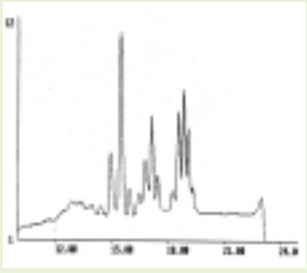
A HPLC method was standardized and a library of chromatograms was constructed using reference strains of mycobacteria.

The chromatographic library was evaluated with known clinical and environmental isolates of mycobacteria and compared with the programme evolved by the Mycobacteriology Division, CDC, Atlanta.

M.bovis ***M.vaccae*** ***M.simiae***







Examples of chromatograms showing 1, 2 and 3 clusters of peaks are shown in the above figures. The study showed that all the reference strains gave chromatograms similar to the published reports and the known clinical and environmental isolates were also confirmed correctly by HPLC.

Despite the high initial investment for the complete HPLC system, the low recurring expenditures makes this technique a method of choice and has the potentials to be employed routinely for the speciation of mycobacteria.

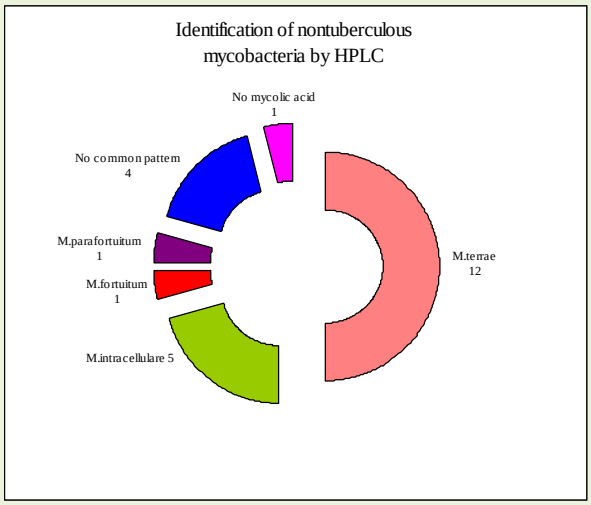
Characterisation of *Mycobacterium terrae*

M.terrae complex are the most frequently isolated mycobacterial species from South Indian BCG trial area besides *M.avium* complex and *M.fortuitum* complex. Although they rarely cause disease in humans, natural sensitization by these organisms may have effect on immune response to skin test antigens, BCG vaccination and mycobacaterial infection.

In continuation with earlier studies on characterization of *M.avium* complex and *M.fortuitum* complex the following experiment was done. Twenty four isolates that were previously identified as *M.diernhoferi* by biochemical method were subjected to mycolic acid analysis by HPLC.

Further characterization was done by lipid analysis using GC-MS at Lund University, Sweden by Dr.Lennart Larsson. *M.terrae* complex organisms are characterized by the presence of Tuberculostearic acid (TBS), 2-eicosanol and trace amounts of hexacosanoic acid. Efforts are underway to carry out pulse field gel electrophoresis for intra-species differentiation of these organisms.

Twelve isolates gave chromatograms comparable with M.terrae complex and others were identified as M.intracellulare, M.fortuitum, M.parafortuitum. Three of the isolates gave pattern that did not match with any of the existing chomatograms and one isolate did not give any mycolic acid peak. Eight of these isolates were cross checked at CDC, Atlanta for mycolic acid analysis to confirm our results.



Evaluation of microplate alamar blue assay for determination of *in vitro* activity of clarithromycin and rifabutin against *M. avium* complex

M.avium complex (MAC) are the most frequent isolates among the nontuberculous mycobacteria excreted repeatedly from patients with abnormal chest X-rays. Although these organisms are innately resistant to the antituberculosis drugs, they are shown to be susceptible to the recently introduced macrolide antibiotic, clarithormycin and the rifamycin derivative, rifabutin. Apart from the conventional susceptibility testing methods (BACTEC radiometric and proportion susceptibility test9PST), a new rapid technique called microplate alamar blue assay (MABA) was evaluated. MABA is a colorimetric broth microdilution technique which utilises an oxidation-reduction dye as an indicator of bacterial growth.

In the present study, the *in vitro* activity of clarithromycin and rifabutin was determined against 52 clinical and environmental isolates of MAC. The results are given in the following table:

Clarithromycin and rifabutin show good activity against MAC when tested by all the three methods. The MABA has good agreement with the BACTEC method (92% and 98% for clarithromycin and rifabutin respectively), while PST shows less agreement (87% and 80% for clarithromycin and rifabutin respectively). This could be due to protein binding and longer incubation followed in the PST technique.

Susceptibility of clarithromycin and rifabutin against MAC and total agreement between different tests

	Clarithromycin %	Rifabutin %
Susceptibility BACTEC	100	92
PST	94	80
MABA	92	94
Total Agreement BACTEC vs MABA	92	98
PST vs MABA	87	80

MABA can be employed for the screening of drugs for antimycobacterial activity since it is economical and rapid.

AFB smear results using two sedimentation agents for sputum samples

A total of 534 sputum samples were collected from 230 pulmonary tuberculosis (PT) patients attending the Government Hospital, Thiruvallur and Peripheral Health Centre, Periyapalayam, where Revised National Tuberculosis Control Programme (RNTCP) is being implemented and studied. The samples were immediately subjected to direct smear (DS) examination for AFB by the Ziehl Neelsen method. The smears were read by the laboratory technicians at the respective centres and the results were reported as per the RNTCP guidelines. The samples received on Mondays, Wednesdays and Fridays were gently mixed with 5 to 10ml of sodium hydroxide-ammonium sulphate (SAS) reagent. The sputum samples received on Tuesdays, Thursdays and Saturdays were treated with phenol-ammonium sulphate (PAS) reagent. The samples were occasionally shaken and left at room temperature over night or till they were taken up for sediment smear (SS) preparation. The contents of the sputum got sedimented after an overnight storage. After decanting the clear supernatant, a drop of the sediment was smeared over a clean glass slide. The smear was air-dried, heat fixed, stained by the Ziehl Neelsen method and read at Tuberculosis Research Centre, Chennai, by proficient readers who were unaware of the DS results. A total of 295 samples by the SAS method (Table-1) and 239 samples by the PAS method (Table-2) were investigated.

Table 1.
Comparison of DS and SS in SAS processed samples.

		DS				
	Neg	Sc	1+	2+	3+	Total
Neg	227	0	1	1	1	230
Sc	4	0	2	0	0	6
SS 1+	2	2	3	1	1	9
2+	0	0	4	7	4	15
3+	0	0	5	5	25	35
Total	233	2	15	14	31	295

Table 2.
Comparison of DS and SS in PAS processed samples.

		DS				
	Neg	Sc	1+	2+	3+	Total
Neg	169	0	0	0	0	169
Sc	15	0	0	0	0	15
SS 1+	7	0	9	1	0	17
2+	3	0	4	9	0	16
3+	0	0	3	8	11	12
Total	194	0	16	18	11	239

The number of sputum samples positive by the SAS method were 65 & 62 in the SS and DS respectively, while the corresponding numbers were 70 & 45 by the PAS method. All the 25 specimens positive in the DS were also positive in SS using the PAS reagent. Three of the specimens positive in DS were missed in SS using the SAS reagent. There were 25 extra positives in SS using the PAS reagent while 6 were extra positive in SS using the SAS reagent.

The results of the study reveal that SS of PAS method is more sensitive in picking up the positives than the SS of SAS method. Phenol is a sterilising agent. The sedimented sputum samples are entirely different from the original sputum sample. The smears can be prepared with ease and less time is required to grade smears. PAS seems to be a better reagent for sedimentation of sputum samples for AFB smear microscopy.

Mutations in the 81-bp RRDR of *rpoB* gene of *M.tuberculosis* clinical isolates

Rifampicin (RIF) resistance serves as a surrogate marker for the detection of MDR-TB as 90% of RIF resistant (RIF^r) isolates are also isoniazid-resistant (INH^r). RIF interferes with transcription and elongation of RNA by binding to the DNA-dependent RNA polymerase. It was observed that resistance to RIF follows a "single-step" high-level resistance pattern in which the mutants occur spontaneously at a frequency of 10^{-9}. Genetic basis for RIF resistance in approximately 95% of the cases is due to mutations in an 81-bp Rifampicin Resistance Determining Region (RRDR) of the *rpoB* gene corresponding to 507-533 codons (*E.coli* numbering system) that codes for the beta-subunit of RNA polymerase of *M.tuberculosis*.

In the present study, mutations in the 81-bp RRDR of the *rpoB* gene were studied in 50 *M.tuberculosis* clinical isolates (44 resistant and 6 sensitive) isolated from various parts of India. None of the sensitive isolates showed any mutation. Single-base substitutions and deletion in a total of 53 mutations of 18 different kinds were observed in the RRDR of *rpoB* gene of resistant isolates. A single resistant isolate did not show any mutation. Three novel mutations and 3 new alleles within the RRDR along with 2 novel mutations outside the RRDR are reported in this study. These sequences were submitted to the EMBL Nucleotide Sequence Submissions and the following accession numbers obtained: AJ297922, AJ297923, AJ297924, AJ297925, AJ297926, AJ297927, AJ297928 and AJ297929 (Confidential till November, 2001).

Rapid drug susceptibility testing of *Mycobacterium tuberculosis* cultures by luciferase reporter phage assay using phAE85 and phAE129

A total of 66 clinical isolates of *M.tuberculosis*, 3 quality control strains and a reference strain, namely, *M.tuberculosis* $H_{37}Rv$ were tested for their susceptibility to 1 and 2 µg of isoniazid (H) and rifampicin (R) per ml, using luciferase reporter phage (LRP) assay. The original procedure for LRP assay was to grow the isolates in 7H9 broth for 7 days and exposing the same to the action of the drugs for 48 hrs. Modifications to this protocol were found to be necessary to overcome sample contamination. In one protocol, supplemental vancomycin (10 µg/ml) was added to 24 hr-old cultures growing in malachite green (2.5 µg/ml) containing 7H9 broth, wherein the drug susceptibility test results were made available on the 7th day (Regular LRP). The 2nd method (Direct LRP) relied on a short incubation of 3 days of a higher inoculum prepared directly from the LJ medium in drug-free and drug containing 7H9 media and the results were made available on the 3rd day itself. Two phage constructs, namely phAE85 (TM4 based) and phAE129 (D29 based) were used both in Regular and Direct LRP formats.

Fig.1 The percentage of drug resistant cultures detected by LRP assays as compared to standard indirect sensitivity testing

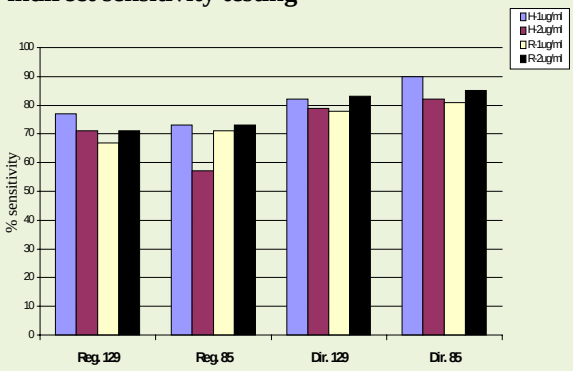


Fig. 2 The percentage of drug sensitive cultures detected by LRP assays as compared to standard indirect sensitivity testing

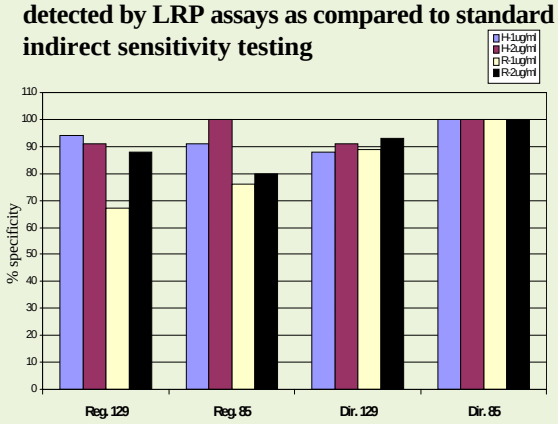
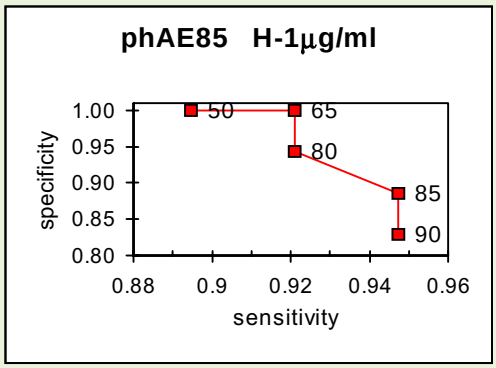
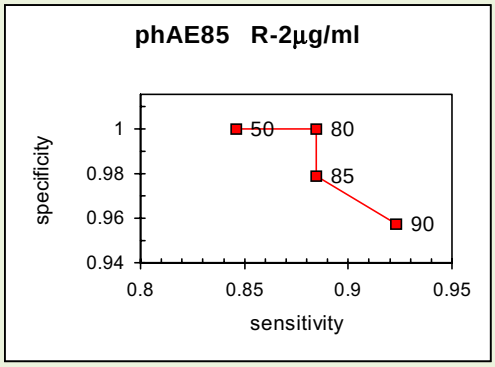


Fig 3. Effect of various inhibition breakpoints on the specificity and sensitivity of Direct LRP using phAE 85





Among the four LRP procedures used, results of Direct LRP sensitivity assay using phAE85 showed a better correlation with those of the conventional susceptibility test (fig.1&2). With a growth inhibition index breakpoint of 50%, the agreement of the Direct LRP results with the conventional method was 95%, both for H (1µg/ml) and R (2µg/ml). While all the drug susceptible isolates were correctly identified by this test, the sensitivity of the assay to detect drug resistance was 90% and 85% for H and R respectively. The level of sensitivity increased to greater than 92% with both the drugs, if an inhibition index breakpoint of 90% was used (fig.3).

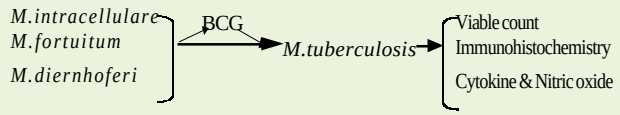
These results suggest that Direct LRP assay with phAE85 can be used as an alternate method for the conventional sensitivity test for *M.tuberculosis*.

Effect of continuous exposure of nontuberculous mycobacteria on the protective immunity induced by BCG in mice

Due to the varied efficacy of BCG in different parts of the world it was proposed that natural sensitization by nontuberculous mycobacteria (NTM) might influence the protective immunity induced by BCG. Many studies were conducted in our centre to test this hypothesis. All those studies were done on guinea pigs and NTM was given at one time point and the effect of NTM on BCG was studied by skin test reactions and viable count studies. The results showed that oral exposure to NTM did not interfere with subsequent immune response to BCG, while certain amount of modulation of immune response to BCG was noted at the later stages of infection when NTM was given intradermally or subcutaneously.

This study was designed in mouse model and more sensitive methods for the measurement of immune response were also employed.

Study design



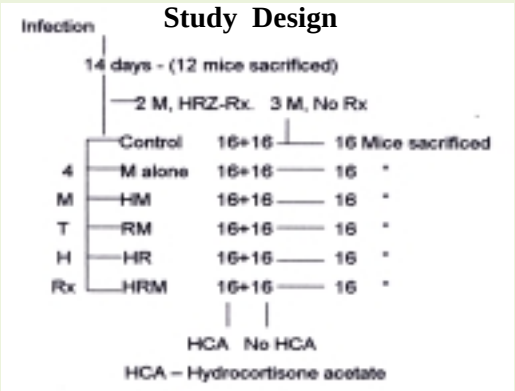
Groups of mice were exposed to NTM namely, *M.intracellulare*, *M.fortuitum* and *M.diernhoferi*, the most common mycobacterial species found in the environment and this was followed by BCG immunization. Proper control groups were also included. The effect of NTM on the protective immunity induced by BCG was studied by challenging the mice with *M.tuberculosis* and by performing viable count studies, measurement of cytokines (INF-γ, IL-4 and TNF-α), nitric oxide and immunohistochemistry studies. The results are being analysed.

Dormancy in tuberculosis: A newer approach of treatment in the murine model

Results of the previous experiment given in the 1998 annual report on the action of metronidazole (M) in combination with isoniazid (H) and rifampicin (R) on persisting organisms in experimental murine tuberculosis revealed that M showed an additive effect when combined with R in reducing the $\log_{10}$ CFU of *M.tuberculosis* in both the lungs and spleen at the end of 3 months of treatment. When M was combined with H, this effect was observed only in spleen and not in lungs. Since only few animals were included in each group of this experiment the differences attained were not statistically significant. Meanwhile conflicting observations on the action of M were published elsewhere. We repeated the experiment with an improved study design including more numbers of animals in each group.

According to the study design given below, a total of 312 Swiss, female mice were infected with 10^7 organisms by tail vein. Treatment was started 14 days after infection according to the following protocol.

Study Design



Treatment Protocol

Group	Drug	Dose(mg/kg body wt.)	Duration in Months
1	(Control)	No Rx	4
2	M	100	4
3	HM	25+100	4
4	RM	10+100	4
5	HR	25+10	4
6	HRM	25+10+100	4

Before the start of treatment, 12 mice were sacrificed and VC was set up from spleen and lungs. The remaining 300 mice were treated with HR & pyrazinamide (Z) (H-25 mg/kg, R-10 mg/kg, Z-1000 mg/kg) for 2 months. At the end of 2 months, 12 mice were sacrificed and VC was set up from spleen and lungs. The remaining 288 mice were divided into 6 groups containing 48 mice each. The first group served as control and did not receive any treatment. The remaining five groups received M, HM, RM, HR and HRM for 4 months.

To study the activity of M after 4 months of treatment, 16 mice from each group were given hydrocortisone acetate (HCA) by s/c injection for 7 days (1mg/day/mouse), after which all the 16 animals treated with cortisone and another 16 animals without cortisone from each group were sacrificed, the spleen and lungs were homogenised with sterile distilled water and were processed to check the presence of tubercle bacilli in spleen and lungs by BACTEC 460 Radiometric system and also by inoculating into Kirchner's liquid medium. Similarly, at the end of 3 months after stopping the treatment 16 mice were sacrificed from each group and the presence of tubercle bacilli in spleen and lungs were measured.

The experiment has been completed and the results are being analysed.

FAST plaque TB assay for rapid diagnosis of Tuberculosis

The kit for FAST plaque TB assay for tuberculosis diagnosis, supplied by Biotech Laboratories, UK was evaluated using sputum specimens of tuberculosis patients admitted to various ongoing studies. A total of 73 specimens were processed by NALC-NaOH method following the procedure given in the package insert. The results when compared with conventional smear and culture results showed a poor agreement with very low sensitivity and specificity.

A modification of the original procedure was attempted, wherein the sputum specimens processed by Petroff's procedure as well were tested in addition to NALC-NaOH deposits. In 109 sputum specimens processed by Petroff's method, an agreement of 72% was observed when compared with smear results. Though the sensitivity of the assay was moderate (65%), the specificity was as high as 90%. When compared with culture results, the accuracy and sensitivity of the assay increased, though the specificity decreased slightly (Fig.1&2).

The modification of the procedure did not improve the results with specimens processed by NALC-NaOH. Though the accuracy and sensitivity of the assay were high, the specificity was very poor when compared to both smear and culture results.

Fig.1

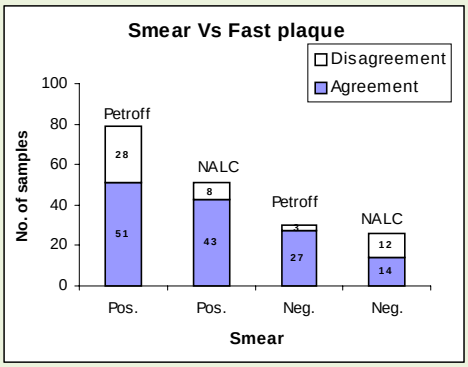
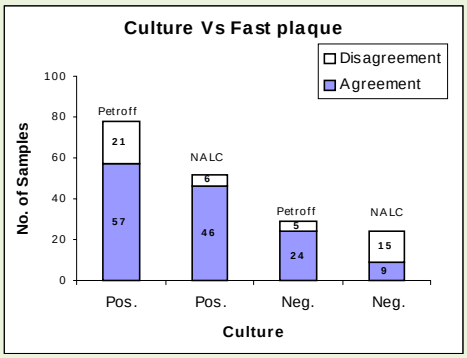


Fig.2



The assay performed with Petroff's deposits is highly specific and moderately sensitive. With NALC-NaOH deposits, the sensitivity of the assay is substantially high but at the expense of specificity. It is proposed to strengthen this assay by supplementing antibiotics to the Petroff's deposits with overnight incubation prior to the assay.

Staff List

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Sulochana Somasundaram, M.S.(MLT.),D.M.L.T.

Awards

Prize awarded for the best presentation at the International symposium on 'Luminescence and its Applications' held at Baroda during Feb. 2000 - **Dr.Vanaja Kumar**

Elected as Fellow of the National Academy of Medical Sciences during Feb. 2000 - **Dr.C.N.Paramasivan**

Capacity Building

Doctor of Philosophy

Thesis in Progress

Mr.G.Kubendiran -Evaluation of the activity of anti-tuberculosis drugs and drugs that act on dormant tubercle bacilli under *in vitro* and *in vivo* conditions.
- The Tamil Nadu Dr.MGR Medical University

Ms.Daisy Vanitha - Characterisation of Non tuberculous Mycobacteria (NTM) and their role in immune response in experimental tuberculosis. - The Tamil Nadu Dr.MGR Medical University

Mrs.Sulochana Somasundaram - Analysis of molecular basis of fluoroquinolone resistance in mycobacteria. - University of Madras

Ms.C.Mani - Development of a molecular method for the rapid detection of drug resistance in mycobacteria. - The Tamil Nadu Dr.MGR Medical University

Master of Science

Mrs.Sulochana Somasundaram - Sankara Nethralaya, Chennai along with Birla Institute of Technology, Pilani (Completed).

Mr.Prabhakaran - Post Graduate Institute, Chandigarh (Completed).

Mrs.N.S.Gomathi & Mrs.Gomathi Sekar - Prince Venkateswara College of Arts and Science, Gowrivakkam (In progress).

Laboratory Technician Course

Mrs.Rajeswari Balasubramaniam -CLTRI, Chengalpattu (Completed).

Mr.A.Shyam Sundar - CLTRI, Chengalpattu (In progress)

Participation in Conferences / Workshops / Symposia

National

I[st] Indo French Symposium on 'Multiple Drug Resistance and Emerging Diseases.' held at Indian National Science Academy, New Delhi during Feb. 1999 - "Multi drug resistance in Tuberculosis." - **Dr.N.Selvakumar**

Conference of Pathologists and Microbiologists of Vidharba region -'Laboratory diagnosis of Tuberculosis - a realistic approach under Indian condition' held at Sewagram, Wardha during Apr. 1999
- **Dr.C.N.Paramasivan**

Symposium on 'Tuberculosis – The Indian Perspective' organised by ASTRA Zeneca, Bangalore during June,1999 - "MDR TB in India and its implications" - **Dr.C.N.Paramasivan**

Symposium on 'Microbiology in the New Millennium' organised by Association of Microbiologists of India. (Madras Chapter) during Dec. 1999. (Panel member) - **Dr.N.Selvakumar**

54th National Conference on Tuberculosis and Chest Diseases held at Patna during Dec. 1999 - "An overview on MDR-TB in India" (delivered this oration at the invitation of TB Association of India) - **Dr.C.N.Paramasivan**

5th International Conference on emerging infectious Diseases in the Pacific rim, held at Chennai, Jan. 2000 - "Some issues relating to pathogenesis of tuberculosis" - **Dr.C.N.Paramasivan**

Symposium on 'Advances in the pharmacology of anti infective therapy' - organized by Astra Zeneca Research Foundation, India and International Society for Anti-Infective Pharmacology held at Indian Institute of Science, Bangalore during Jan. 2000 - "Prediction of clinical efficacy of anti-tuberculosis regimens from *in vitro* and *in vivo* studies." - **Dr.C.N.Paramasivan**

One day seminar on 'Luminescence and its applications' held at Annamalai University, Chidambaram during Aug. 2000 - "Bioluminescence assay: What is new?" - **Dr.Vanaja Kumar**

Indian Association of Medical Microbiologists, Tamil Nadu and Pondicherry Chapter held at SRMCRI, Porur, Chennai during Aug. 2000. (Chairperson for a session) - **Dr.N.Selvakumar**

Ranbaxy Science Foundation's 7th Round Table Conference on 'Tuberculosis' held at India Habitat Centre, New Delhi during Sept. 2000 - "Prevalence and Microbiology of MDR-TB" - **Dr.C.N.Paramasivan**

International Symposium on 'Luminescence and its Applications' held at Baroda during Feb. 2000 - "Bioluminescence and luciferase reporter phage assay"(Invited talk), "Biomedical application of luminescence; luciferase reporter phage assay for rapid drug susceptibility testing of *M.tuberculosis* isolates." (Poster cum oral presentation) - **Dr.Vanaja Kumar**

International

ASM conference on 'Tuberculosis: Past, present and future' held at New York during June 2000 - **Dr.Vanaja Kumar**

Membership in Expert Committees & Special Assignments

Temporary Advisor, WHO (SEARO) to conduct Regional Training Workshop on 'Laboratory Methods of TB Control' at Jakarta, Indonesia during Apr. 1999. - **Dr.C.N.Paramasivan**

Temporary Advisor, WHO (SEARO) - Participation 'Informal consultation to review SOPS on laboratory diagnosis of HIV associated infections' held at Bangkok during Sept. 1999 - **Dr.C.N.Paramasivan**

Invited to participate in 'TB modular course' - sponsored by the WHO, Geneva, held at ALERT,Addis Ababa during Oct.,1999 - **Dr.C.N.Paramasivan**

Temporary Advisor: WHO (SEARO) Participation 'Inter-country Training on Laboratory Diagnosis of HIV and opportunistic Infections' held at WHO collaborating centre for Training and Research on HIV/AIDS, Dept. of CDC, Bangkok, Thailand during Dec. 1999 - **Dr.C.N.Paramasivan**

Expert Member: - Work Group Meeting convened by the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL) - co sponsored by the WHO and International Union Against TB and Lung Disease (IUATLD) to 'Develop Consensus and Guidance on External Quality Control for microscopy network in low and middle income countries' held at Amsterdam, The Netherlands during Mar. 2000 - **Dr.C.N.Paramasivan**

Temporary Advisor: WHO (SEARO) Intercountry workshop – Drug Resistance Surveillance in Tuberculosis at Bangkok, Thailand during Aug. 2000 - **Dr.C.N.Paramasivan**

Special Invitee to Scientific Advisory Committee Meeting of National Institute of Occupational Health, Ahmedabad during Sept. 2000 - **Dr.N.Selvakumar**

Participated in a meeting on Quality control measures in the laboratory diagnosis of Tuberculosis held at CDC, Atlanta, USA. during Sept. 2000 - **Dr.C.N.Paramasivan**

Participated in 'Inclen Global Meeting' on drug resistance surveillance held at Bangkok, Thailand during Oct. 2000. - **Dr.C.N.Paramasivan**

Training Programmes

Training in smear microscopy, culture, drug sensitivity & quality control was offered to many international (Myanmar, Bangladesh) and national staff & students for a period ranging from 1 week to 3 months.

Advocacy

Refresher course for College Teachers - Uptake in Microbiology. Theory and Practice - Molecular Basis of drug resistance in *M.tuberculosis* held at University of Madras, Chennai during Jan.1999 - **Dr.N.Selvakumar**

All India Radio, Chennai. (Talk) - Bacteriological investigations in Controlled Clinical Trials and achievements in Bacteriology laboratory in the last 40 years - at 8.45 pm on 19th Oct.1999 - **Dr.N.Selvakumar**

CME - 2000 on 'Current concepts in tuberculosis for Clinical Microbiologists' - jointly organised by the National Tuberculosis Institute, IAMM, Karnataka Chapter & Medical Education and Research Trust, Bangalore, held at NTI, Bangalore during Jan.2000 - "Conventional and rapid culture methods of mycobacteria".
- **Dr.C.N.Paramasivan**

Contact Persons

Drug resistance surveillance
Quality control & quality assurance
Early Bactericidal Activity
Dormancy
Environmental mycobacteria
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Training Programmes
Early diagnosis of tuberculosis
Molecular mechanisms of rifampicin resistance
Model DOTS Project
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Luciferase phage reporter assay
Development of newer phage reporter systems
Evaluation of diagnostics
Dr.Vanaja Kumar
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IMMUNOLOGY

OVERVIEW

The Department of Immunology focuses on the biological, immunological and molecular biological aspects of mycobacterial infections and helminthic diseases. The department is involved in studies on basic pathogenic mechanisms that may lead to better diagnostic tools and development of vaccines and other immune interventions for prevention and control of infection and disease. The department has adopted a multidisciplinary approach that includes immunology, molecular biology and epidemiology to study tuberculosis. Immunologic studies focus on genetic regulation of the immune response, the role of both HLA and non-HLA polymorphisms, and cytokines involved in the immune responses to tuberculosis. Antigen purification and immunodiagnosis are other major areas. More recently, in field based trials, the department is using molecular epidemiology to study the impact of DOTS implementation in a rural area. Another recent initiative is the study of immunological consequences of co-infection with tuberculosis and helminths.

NEW INITIATIVE

Molecular Epidemiology
Regulation of Gene Expression in *E.coli* & mycobacteria
Apoptosis in tuberculosis

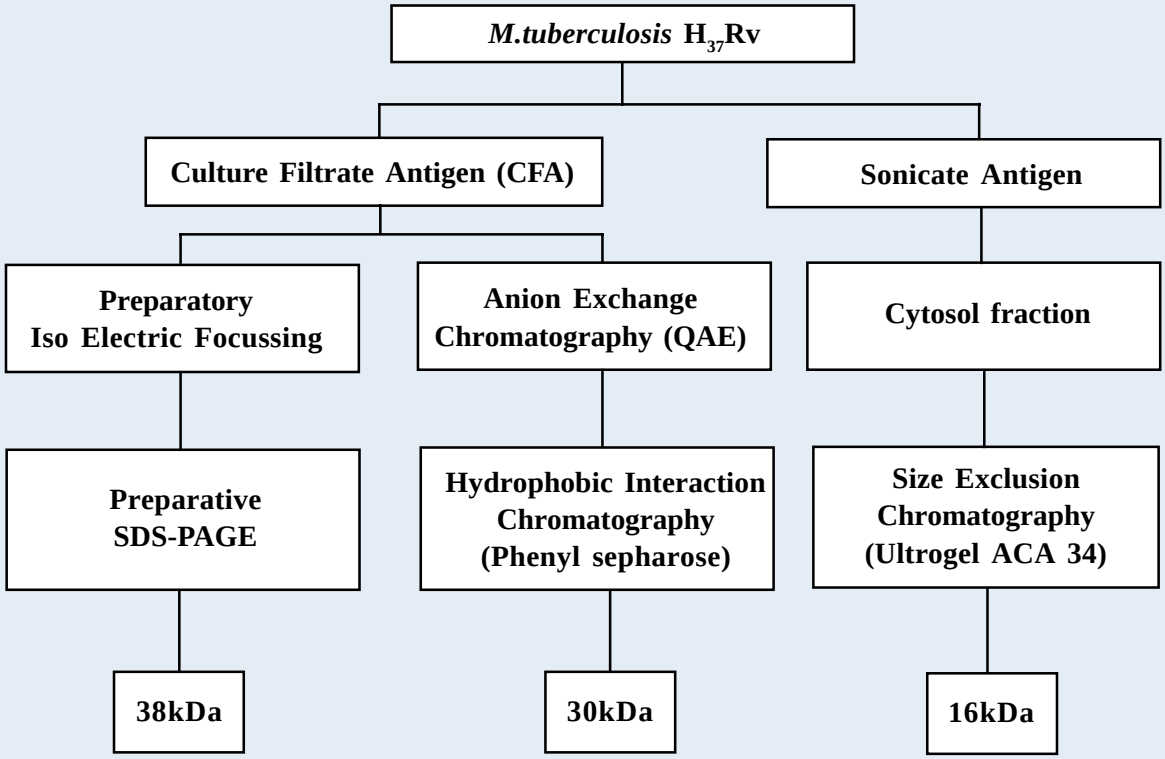
RESEARCH FOCUS

Immunodiagnosis
Immunogenetics
Gene Expression
Cellular Immunology

Purification of *M.tuberculosis* antigens and Immunodiagnosis

Rapid and early diagnostic methods play an important role in the control of tuberculosis. Isolation of individual antigens of *M.tuberculosis* is an important pre-requisite for development of specific diagnostic assays. Among all the antigens, the secreted antigens of *M.tuberculosis are* important, since they are the earliest which the host immune system encounters and has an opportunity to respond. We isolated and evaluated two actively secreted antigens, 38 & 30kDa and one cytosolic antigen, 16kDa for their potential use in diagnostics.

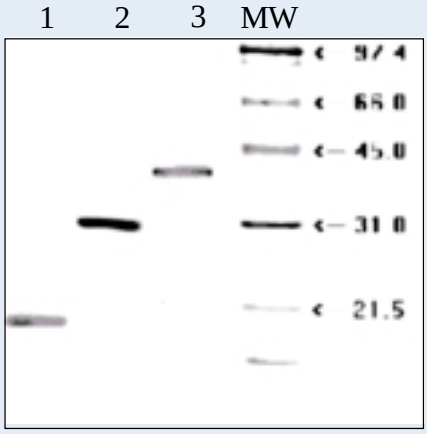
Fig.1 Purification of antigens



We purified 38 and 30kDa antigens from *M.tuberculosis* culture filtrate, and the 16kDa antigen has been purified from *M.tuberculosis* cytosol, using physicochemical methods (Fig.1). These antigenes were evaluated for detection of serum IgG, IgA and IgM antibodies by ELISA for their potential usefulness in the diagnosis of tubeculosis. The purified antigens are shown in Fig.2

The serum antibody levels of isotype IgG, IgA and IgM were determined in all these sera using ELISA. The cut-off point was chosen as Mean + 2 S.D. of normal sera O.D.

Fig.2 Purified Antigens



Lane 1: 16kDa Lane 3 : 38kDa
Lane 2: 30kDa MW : Mol. Wt. marker

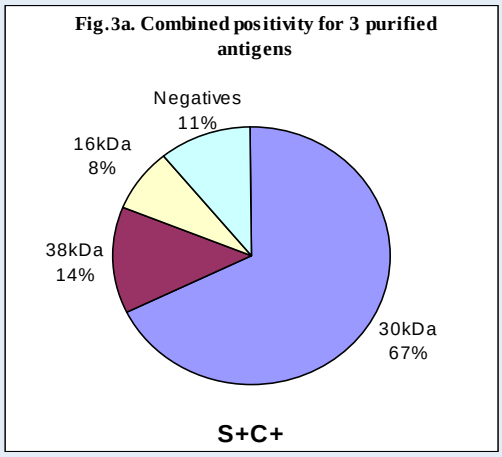
For the polar groups (S+C+ and NHS), the sensitivity and specificity obtainable for each of the isotype and combination of isotypes have been worked out. The range of values for 38, 30 and 16 kDa have been summarized in the accompaning table.

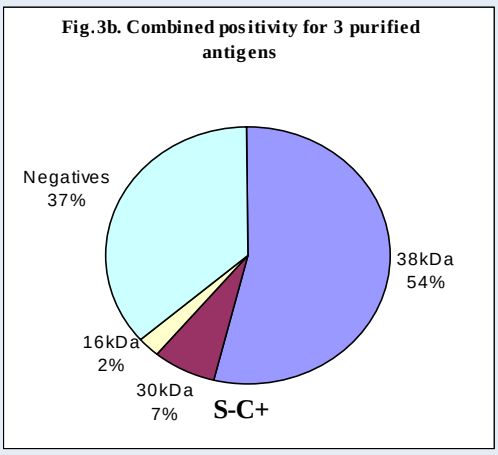
Diagnostic test characteristic of ELISA using the combination of the 3 purified antigens was calculated as compared to single antigens. These results are depicted in Fig. 3a & b.

Polyethylene glycol precipitation of the circulating immune complex (CIC) in sera was carried out and the antibody level in the CIC was also assessed by ELISA. The CIC bound 38kDa antibodies were measured. The results are shown in Fig.4. Using 30 and 16kDa antigens, high positivities (92.5 – 97.5% in S+C+; 85.4 – 100% in S-C+ and 64–68.5% in S-C-) were achieved. The specificity of the assay remained high at 95 - 96.6%.

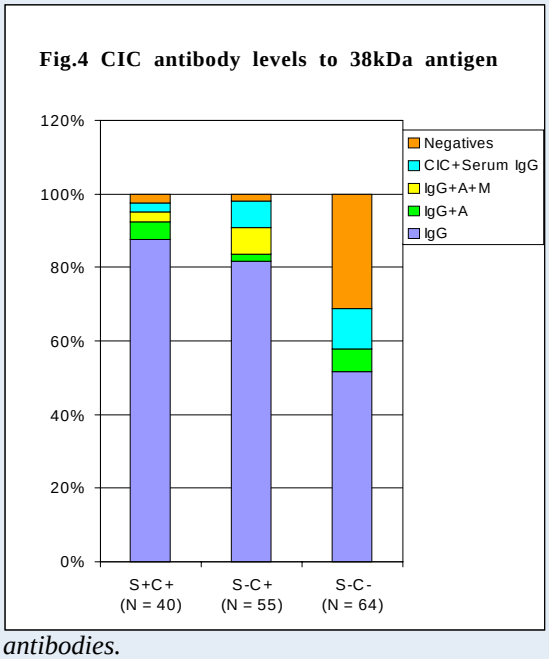
Table - Range of ELISA positivity in the polar groups for 38, 30 and 16kDa antigens

ISOTYPE	S+C+(n=175)		NHS(n=150)	
	No. +ve	% SEN	No. +ve	% SP
IgG	106-118	61-67	0-2	99-100
IgA	52-91	30-52	4-6	96-97
IgM	18-25	10-14	6-9	94-97
IgG+IgA	119-144	68-82	4-6	96-97
IgG+IgA +IgM	125-145	71-83	10-15	90-93





Using only the IgG antibody levels, a high positivity of 89.1%(156/175), with a specificity of 98.6%was obtained. Similarly, in S-C+ group also the sensitivity improved but marginally to 63.4%.



Using a panel of 3 antigens, it was possible to obtain a good diagnostic test of 89% sensitivity and 99% specificity. CIC bound antibodies promise to be an even better diagnostic tool in the detection of smear positive and negative tuberculosis.

95%, 90.9% and 57.8% positivities were achieved for S+C+, S-C+ and S-C-groups for detection of CIC bound immunoglobulins. The positivity further increased to 97.5%, 98.1% and 68.7% in the 3 groups respectively by combination of serum IgG and CIC antibody results. None of the CIC of NHS was positive. Even the 8/60 sera from patients of non-TB lung diseases were negative for CIC bound antibodies.

HLA-genotyping : DNA typing in pulmonary tuberculosis patients and control subjects

Susceptibility to tuberculosis has been suggested to be multifactorial. Though environmental and socio-economic factors are primarily related, numerous studies have emphasized the importance of host genetic factors on resistance and hereditary susceptibility to tuberculosis. The host genetic factors may be divided into Major Histocompatibility Complex (MHC) genes/Human Leucocyte Antigens (HLA) and non-MHC / non-HLA genes. Our studies are focussed on :

i) Identifying a gene or multicandidate genes associated with susceptibility to TB which may serve as genetic marker(s) to predetermine the development of the disease. Individuals positive for such genetic markers would be members of a high risk group and would be useful for screening purposes in an endemic area.

ii) understanding the role played by the host genes that are associated with susceptibility or resistance to *M.tuberculosis* in infection / disease development which may have a role in better management of the disease.

We had earlier shown that HLA-DR2 is associated with susceptibility to pulmonary tuberculosis. An increased frequency of HLA-DR2 was seen in the pulmonary tuberculosis patients than control subjects. DNA typing of HLA-DR2 sub-types using sequence specific oligonucleotide probes (SSOP) revealed that DRB1 *1501 was significantly associated with susceptibility to tuberculosis. However, a trend towards decreased frequency of DRB1 *1502 and *1503 was seen in patients as seen in the following table.

Percentage genotype frequencies of HLA-DR2 alleles in pulmonary TB patients and controls

HLA-DR2 alleles	Controls (n=36)	PTB patients (n=72)	
DRB1 *1501	30.5	53.2*	*p=0.04
DRB1 *1502	52.8	35.1	
DRB1 *1503	16.7	9.1	
DRB1 *1601	0.0	0.0	
DRB1 *1602	0.0	2.6	

The present study suggests that DRB1 *1501 is the main allele of the HLA-DR2 subtypes that is associated with susceptibility to pulmonary tuberculosis.

HLA-DR2 phenotype and plasma lysozyme in pulmonary tuberculosis

Lysozyme, one of the lysosomal enzymes of monocytes / macrophages is involved in the microbicidal activity (innate immune mechanism). It causes lysis of Gram-positive and some Gram-negative bacteria by specific cleavage of the b1-4 linkage between N-acetyl glucosamine and N-acetylmuramate in the peptidoglycan backbone of bacterial cell walls. Binding of lysozyme to carbohydrate moiety such as lipopolysaccharide (LPS) of bacteria has been shown. Since carbohydrate moieties such as lipoarabinomannan and lipomannan have been shown to be present in the cell wall of *M. tuberculosis*, an attempt was made to find out whether lysozyme binds to live *M.tuberculosis*, if so, whether this binding affects viability.

Our earlier work on HLA-DR2 phenotype and plasma lysozyme levels in pulmonary tuberculosis revealed a decreased level of lysozyme in the plasma of HLA-DR2 positive pulmonary tuberculosis patients when compared to DR2 negative patients.The present study was carried out to understand the role of HLA-DR2 and non-DR2 (DR2 negative) genes / gene products on the innate immune mechanism associated with susceptibility to tuberculosis.

The main objectives of the study were to find out the:

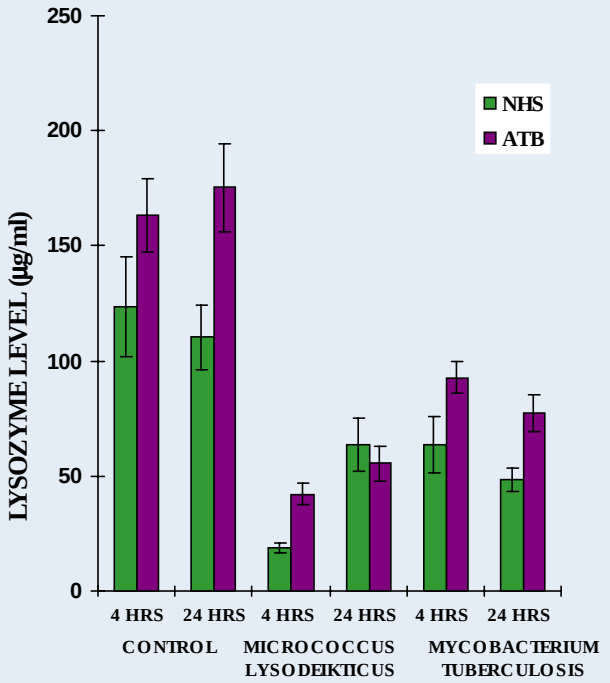
(i) effect of binding of lysozyme on live *M.tuberculosis* H_{37}Rv on its viability and plasma lysozyme levels.

(ii) the effect of live *M.tuberculosis* treated plasmalysozyme of HLA-DR2 and DR2 negative patients with active disease and normal healthy subjects (NHS) on the spontaneous lymphocyte response as well as *M.tuberculosis* culture filtrate antigen induced lymphocyte response in NHS.

The study revealed that plasma samples of active TB patients and NHS treated with live *M.tuberculosis* $H_{37}Rv$ showed decreased lysozyme levels as shown in the accompanying figure. This suggests that lysozyme binds with live *M.tuberculosis*.

The other salient finding of this study was that live *M.tuberculosis* treated with plasma samples of normal subjects and pulmonary TB patients for various time points (4 and 24 hrs) showed a trend towards decreased colony counts when compared to the 0 hr colony count, suggesting that macrophage lysosomal enzymes such as lysozyme plays a role in the innate immunity against *M. tuberculosis* infection. Moreover, plasma of HLA-DR2 positive normal healthy subjects treated with live *M. tuberculosis* (4 hrs) enhances the spontaneous lymphocyte response as well as antigen induced lymphocyte response in HLA-DR2 positive individuals than non-DR2 individuals, showing that binding of lysozyme may induce live *M. tuberculosis* to release some cell wall associated antigens that are liberated due to the hydrolytic action of lysozyme (along with other hydrolytic enzymes such as β glucuronidase etc.) on the carbohydrate.

Fig. Binding of plasma lysozyme on live *M.tuberculosis*



This study suggests that lysozyme plays a role along with other lysosomal enzymes in the innate immune mechanism against *M. tuberculosis* infection. Initial infection in HLA-DR2 positive normal subjects may be associated with a high responder status and in turn they may become tolerant. This may favour the pathogen to establish the infection.

Compartmentalization of effector immune responses at sites of acute pathology in lymphatic filariasis and tuberculous lymphadenitis

Current paradigms on the pathogenesis of most diseases derive almost exclusively from the extrapolation of studies on circulating peripheral blood mononuclear cells, blood being the most easily accessible component of the immune system. However, it is the actual site of disease that is likely to contain high concentrations of parasite antigen in intimate relationship with aggregates of immune cells, and therefore, immunological investigations at the disease site would provide better insights into the host-parasite interactions and pathogenesis of many parasitic diseases.

There have been a few reports in the past, which provide evidence for the concept of immunologic localization at the site of lesion in parasitic diseases such as onchocerciasis, tuberculous pleuritis and tuberculous meningitis. Lymphatic filariasis and tuberculous lymphadenitis are two other diseases that would provide us an opportunity to investigate whether the localized immune response is indeed a true reflection of the systemic immune response or whether there is a 'compartmentalization' of immune responses to the pathogen in regions of acute pathology.

The study involved enumeration of lymphocyte subsets in blood and lymph nodes of patients with lymphatic filariasis, TB lymphadenitis and non-specific lymphadenitis, determination of the state of activation and functional reactivity of the T cell population, and

analysis of the Th1/Th2 character of the immune response in these two locations based on the cytokine profile.

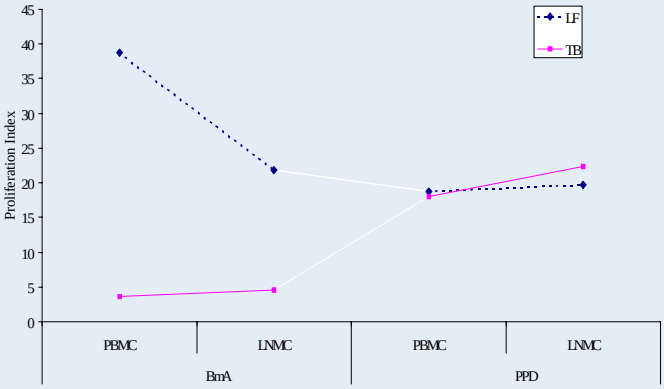
Blood and lymph node sections were obtained from 20 chronic filarial patients who were undergoing surgery for nodo-venous anastomosis and 17 individuals with TB lymphadenitis (confirmed by histopathological examination and bacteriological culture) at the time of lymph node biopsy. 17 individuals with non-specific lymphadenitis (i.e., non-filarial and non-tuberculous lymphadenitis, mainly lymphoma) served as controls for the above two groups.

Lymph nodes were finely chopped in sterile RPMI-1640 medium and passed through a sterile wire mesh in order to obtain single cell suspensions. Lymphocytes were separated from the lymph node cell suspension and heparinized blood by Ficoll-hypaque density gradient centrifugation, and the viable cell yield ascertained by trypan blue dye exclusion method.

Lymphocytes obtained from peripheral blood (PBMC) as well as lymph nodes (LNMC) were analyzed for surface expression of CD3, CD4, CD8, CD16/CD56 and HLA-DR markers using a FACScan flow cytometer after staining with the corresponding fluorescence-tagged monoclonal antibodies.

Comparison of proliferative response of PBMC and LNMC to filarial and tubercular antigens are shown in the following figure:

Comparison of proliferative response of PBMC and LNMC to filarial and tubercular antigens



A significant increase in the percentage of CD4+ cells and a concomitant decrease in the proportion of CD8+ cells was observed in the lymph nodes of individuals with filariasis as well as TB lymphadenitis. This phenomenon could possibly be attributed to sequestration of specific CD4+ T cells at the site of disease in these patients, since the proportion of CD4+ cells in the lymph nodes as well as peripheral blood was similar in the control group.

The proportion of CD4+ T cells and HLA-DR+ T cells in the lymph nodes and peripheral blood of filarial and tuberculous patients are given in the following Table:

CD4+ T cells & HLA-DR+ T cells in lymph nodes & peripheral blood

Group	% CD4+ T cells Blood	Lymph node
Lymphatic filariasis	41.3 ± 2.8	50.5 ± 2.6[a]
TB lymphadenitis	41.6 ± 2.0	51.3 ± 3.0[b]
Non-specific lymphadenitis	52.5 ± 2.2	55.8 ± 3.8
	% HLA-DR+ T cells	
	Blood	**Lymph node**
Lymphatic filariasis	23.9 ± 4.6	33.3 ± 5.7
TB lymphadenitis	18.6 ± 1.8	36.1 ± 4.4[c]
Non-specific lymphadenitis	28.6 ± 1.8	24.8 ± 2.6

The proportion of HLA-DR+ T cells in the lymph nodes of filarial and tuberculous patients was considerably higher than in peripheral blood. This could probably be the consequence of high antigenic load at the site of acute pathology and maximum interaction between immune cells and parasites occurring in this region.

[a] - $p<0.05$, [b] - $p<0.01$, [c] - $p<0.001$

Functional reactivity of the circulating and localized lymphocytes was studied by means of the lymphocyte proliferation assay. Lymphocytes of both filarial and tuberculous patients responded better to parasite-specific antigens in each than to non-specific parasite antigens. This response to specific parasite antigens was more marked in lymph node cells than peripheral blood cells, once again indicating sequestration or preferential expansion of antigen-reactive lymphocytes at the site of pathology (Fig.) The only exception was the lower index obtained for BmA-stimulated LNMC of filarial patients. It is suggested that the BmA antigen used could be contaminated with microfilarial antigens present in gravid females, since microfilarial antigens are known to bring about hyporesponsiveness in parasite antigen specific T cells. This suggestion is validated by the observation that LNMC from these individuals exhibited a very high proliferative response when stimulated with antigens extracted from male filarial parasites alone.

Neither the PBMC nor LNMC of the control subjects responded proliferatively to either filarial or tubercular antigens. This reveals the absence of specific T cells sensitized to these antigens in this group of individuals. The cytokine profile was determined in the local compartment and compared with the systemic cytokine profile in order to see if there was skewing of the immune response towards the Th1 or Th2 type in the two compartments.

It was generally observed that the Th2 cytokine response in both the compartments remained unaltered.However, there was a marked increase in the production of the Th1 type cytokine, interleukin-2, by lymph node cells of filarial patients as compared to peripheral blood lymphocytes in response to the filarial antigen BmA (PBMC = 38.9 pg/ml; LNMC = 457.6 pg/ml). In patients with TB lymphadenitis, LNMC produced measurable quantities of both IL-2 and IFN-γ, but very negligible amounts of all the 3 Th2 type cytokines tested in response to PPD (IL-2 = 256.0 pg/ml; IFN-γ = 523.2 pg/ml). These observations reveal an active Th1 type of immune response in the lymph nodes of individuals with lymphatic filariasis as well as tuberculous lymphadenitis.

The above findings indicate that there is a trend towards localization or 'compartmentalization' of an active, proinflammatory type of immune response in regions of acute pathology in individuals with lymphatic filariasis and tuberculous lymphadenitis. Hence, the immunopathology associated with the above two disease conditions might be the consequence of these localized immune responses.

Differential expression of a unique protein by intracellular *M.tuberculosis* complex

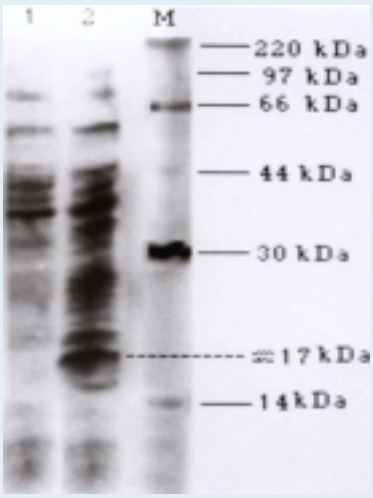
M.tuberculosis is an intracellular pathogen. The macrophage fails to eliminate this pathogen despite a powerful array of antimicrobial defences it puts forth along with other components of the immune system suggesting *M.tuberculosis* adapts itself to the intracellular environment by mounting an appropriate response to ensure its survival. The resistance of *M.tuberculosis* to macrophage destruction involves a number of genes. The protein products of these genes would not only be potential determinants of virulence but also important in cell mediated and protective immune response to *M.tuberculosis* because they are processed and presented by infected macrophages. Identification and characterisation of *M.tuberculosis* proteins would help us to understand the pathogenesis of tuberculosis and in the development of novel drug targets and attenuated vaccines.

We undertook a study to identify protein products which are uniquely expressed by *M.tuberculosis* in the intracellular environment of the guinea pig macrophages *in vitro*. We chose guinea pigs because the tuberculosis disease in this animal model mimics the disease in humans. We examined the pattern of protein synthesis in peritoneal macrophages of guinea pigs following infection with virulent *M. tuberculosis* $H_{37}Rv$ *using* ^{35}S - methionine labelling of the newly synthesized proteins followed by ultracentrifugation and SDS-PAGE.

More than 40 bands were observed both in the cytosolic and membrane fractions from the infected and uninfected macrophages (data not shown). A large number of cytosolic proteins were differently expressed by *M.tuberculosis* infected macrophages *in vitro*.

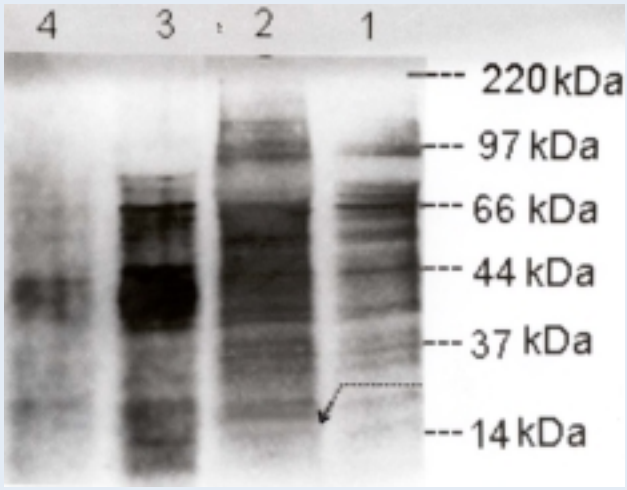
More strikingly one of the proteins of the cytosolic fraction from *M.tuberculosis* infected macrophages was highlighted prominently when compared to the cytosolic fraction from uninfected macrophages (Fig.1).This protein was consistently identified on five occasions, but it was not expressed in a cell free system where *M.tuberculosis* was cultured in RPMI or Dubos broth without macrophages. Fig.2 shows the pattern obtained with sonicate and culture filtrate antigen from ^{35}S methionine labelled bacilli resolved in SDS-PAGE in parallel with the cytosolic fraction from infected and uninfected macrophages.

Fig.1.Unique protein of the cytosolic fraction from *M.tuberculosis* infected macrophages as shown by autoradiogram



Lanes 1 & 2 represent cytosolic fraction from uninfected macrophages and M.tuberculosis infected macrophages respectively. Lane M represents the molecular weight marker. Dotted line points to the unique protein expressed in the M.tuberculosis infected macrophages.

Fig.2.Differential expression of a protein by intracellular *M.tuberculosis* as compared to cell free system



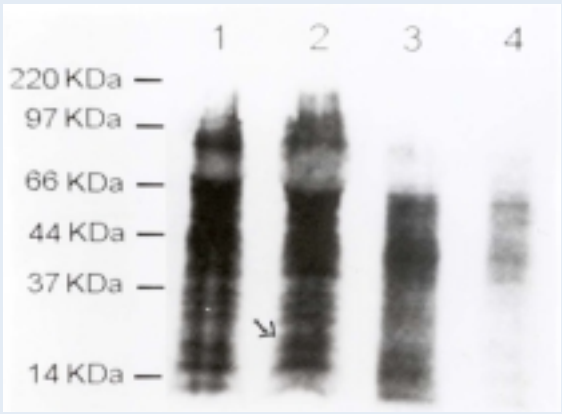
Lanes 1->4 represent cytosolic fraction from uninfected macrophages, infected macrophages, sonicate antigen from M.tuberculosis and its culture filtrate antigen. The arrow points to the unique protein expressed by the M.tuberculosis infected macrophages.

The sonicate and culture filtrate antigen did not show the unique band of molecular weight around 17 kDa implicating that this protein is produced by the *M.tuberculosis* on entry into the macrophages.

Macrophages were infected with heat killed *M.tuberculosis* to determine whether viable bacilli alone are capable of inducing this protein. The cytosolic fraction of the macrophages infected either with heat killed *M.tuberculosis* or heat killed yeast did not express this protein (Fig.3).

Other intracellular pathogens like *Listeria monocytogenes* also did not induce this protein on phagocytosis. *M.bovis* BCG infected macrophages induced this protein to a lower level (data not shown).

Fig.3 Gel pattern of *M.tuberculosis* infected macrophages and macrophages infected with heat killed *M.tuberculosis* and yeast



Lanes 1- 4 show cytosolic fraction from uninfected & infected macrophages, heat killed yeast phagocytosed macrophages and heat killed M.tb phagocytosed macrophages. Arrow shows unique protein expressed by cytosolic fraction from M.tb infected macrophages.

Cloning and expression of *aceA* gene encoding isocitrate lyase from *Mycobacterium tuberculosis*

During the last decade, our understanding of the basic biology of mycobacterial pathogens has benefited greatly from a molecular biological approach. The metabolic processes which lead to dormancy have not been completely explored, though glyoxylate pathway could be one of the possible switch-over mechanisms just prior to or during dormancy.

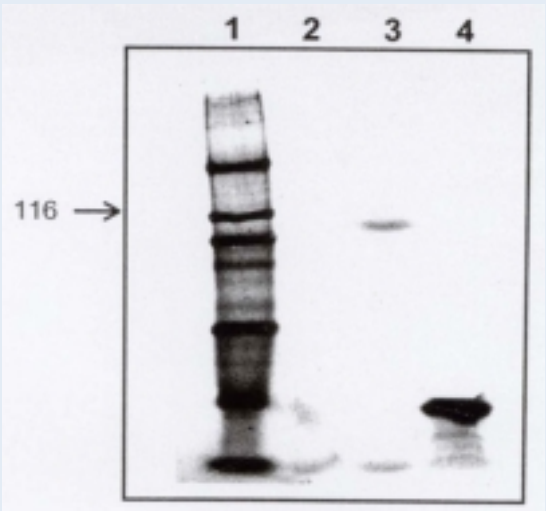
Glyoxylate pathway is one of the important metabolic pathways which bypasses Tricarboxylic acid (TCA) cycle. Isocitrate lyase (ICL) is the first of the two specific enzymes of the glyoxylate cycle. The enzyme reversibly catalyzes the conversion of isocitrate into glyoxylate and succinate. ICL has been demonstrated from *M.leprae*, *M.tuberculosis* $H_{37}Rv$ and other mycobacteria.

ICL activity has been reported to increase with age of the culture in *M.tuberculosis* $H_{37}Rv$ but not in *M.tuberculosis* $H_{37}Ra$ or *M.smegmatis*. Another study reports enhanced glyoxylate cycle enzyme activity under low oxygen tension.

In order to understand whether ICL has a role in dormancy, we cloned and expressed the *aceA* gene of *M.tuberculosis* encoding ICL. Five recombinant clones from a previous partial genomic library of *M.tuberculosis* $H_{37}Rv$ in pGEM4Z were chosen randomly for sequencing. The homology searches performed with the sequence data thus obtained revealed that one of the fragments, TRC9 had high similarity to the cosmid MTCY180 in the predicted *aceA* gene of *M.tuberculosis* in the Sanger Centre database.We therefore decided to study the *aceA* gene in more detail.

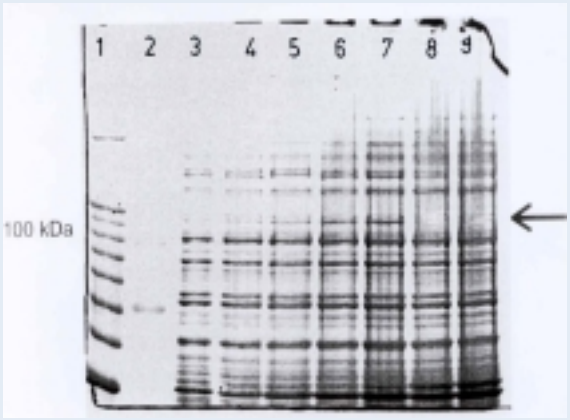
The *aceA* gene was cloned and expressed in Pinpoint XaI vector as biotynilated peptide. The recombinant clone expressed a protein around 100 kDa (Fig.1) which corresponded to the size of the induced fusion product. The *aceA* gene was also expressed using PCR 2.1 system as *lacZ-aceA* gene fusion product. The induced fusion product was seen at 100 kDa (Fig.2).

Fig.1 Western Blot Picture showing expression of *aceA* gene in pinpoint Xa1 vector



E. coli DH5α (lane 2), harbouring pSAP10 (lane3) and pinpoint control vector (lane4) were induced with IPTG, heat lysed proteins were run in a 7.5% SDS-PAGE, along with biotinylated molecular weight marker (lane1), transferred onto a nitrocellulose membrane and detected using the streptavidin – alkaline phosphatase system.

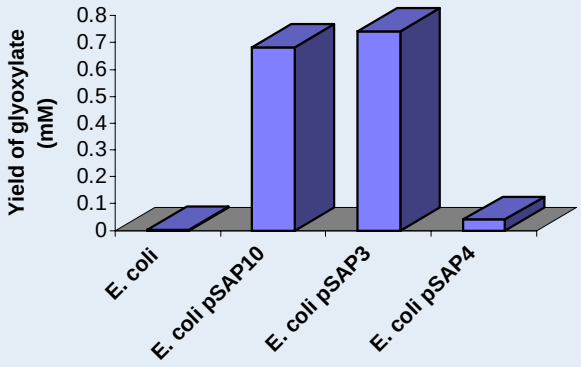
Fig.2 SDS-PAGE showing expression of *aceA* gene of *M.tuberculosis* in pCR 2.1 vector



Proteins from E. coli DH5α harbouring pSAP3 before induction (lane 3) and after 30, 60, 90 & 120 minutes (lanes 4 to 7) of induction with IPTG, E. coli harbouring pSAP4 (lane 8) 120 minutes after induction, and control E. coli (lane 9) 120 minutes after induction were run along with 10 kDa protein ladder (lane 1) and low molecular weight marker (lane 2) in 10% SDS-PAGE and stained with CBB. The arrow mark points to the induced fusion product.

ICL enzyme assay was performed using crude sonicate. This revealed that the protein expressed by both the tac promoter in the PinPoint system and the lac promoter in the pCR2.1 system was functionally active. The yield of glyoxylate obtained after induction of the recombinant clones with IPTG has been shown in Fig.3.

Fig.3 Yield of glyoxylate obtained after induction with IPTG



Studies on HLA and non-HLA gene polymorphism in pulmonary and spinal tuberculosis

Our earlier studies on pulmonary tuberculosis revealed the association of HLA-DR2, Functional Mutant Homozygotes (FMH) of Mannose Binding Protein (MBP) or Mannose Binding Lectin (MBL), and mutant genotype tt of vitamin D receptor genes with susceptibility to pulmonary tuberculosis.

We carried out serological determination of HLA-A, B, DR and DQ antigens in 60 spinal tuberculosis patients and 60 patient contacts to determine whether HLA and non-HLA genes are associated with susceptibility to spinal tuberculosis.We have completed studies on Non-HLA gene polymorphisms such as IL-1 receptor antagonist (IL-1RA), vitamin D receptor (VDR) and tumor necrosis factor alpha and beta(TNF-α & β) gene polymorphisms. Studies on MBL or MBL gene polymorphism (associated with susceptibility to pulmonary tuberculosis) are in progress. DNA typing of HLA-DR genes is also in progress.

Natural Resistance Associated Macrophage Protein-1 (NRAMP-1) gene is the human homologue of the mouse BCG gene and is involved in macrophage activation. It is associated with the susceptibility or resistance to intracellular pathogens such as Leishmania parasites (Lsh) Salmonella (Ity) and some strains of Bacillus Calmette Guerin (BCG).

We investigated the possible role of NRAMP-1 gene polymorphic variants in susceptibility to tuberculosis. We have shown that mutant genotype tt of vitamin D receptor gene region (TaqI polymorphism) is associated with susceptibility to pulmonary tuberculosis in females. BsmI and ApaI gene polymorphisms of vitamin D receptor gene region will be studied in 100 pulmonary-TB patients and 100 control subjects.

The results of the other gene polymorphisms are being analysed.

Immune responses in tuberculous pleuritis: Cell subset profile in blood and pleural fluid of TB pleuritis patients and non-TB pleuritis controls

We had earlier shown that TB pleuritis subjects had an increase in cell proliferation in both blood and pleural fluid (PF) when stimulated with crude mycobacterial antigens. Subsequently we also studied the blastogenic response of these patients against purified mycobacterial antigens, like 10 kDa, 38 kDa and 70 kDa. The blastogenic response to purified antigens was higher than the crude mycobacterial antigens. Further, PF cells showed higher reactivity to purified antigens than the blood cells of the same patients.

To further understand the role of lymphocytes in the pathogenecity and compartmentalization of the disease, we studied the lymphocyte subset profile in both PBMC

& PF cells by FACS using a B-D panel of monoclonal antibodies (Fig.1). Our flowcytometric data suggested that the percentage of $CD3^+$, $CD4^+$ cells and the $CD4^+/CD8^+$ ratio was increased in the PF as compared to that in the blood of the TB pleuritis patients. There was also an increase in the percentage of $CD8^+$ cytotoxic T cells in both blood and PF in the TB pleruitis patients as compared to the control cases (Fig.2).

Fig. 1 Cell subset profile in Blood and Pleural Fluid of 8 TB Pleuritis patients

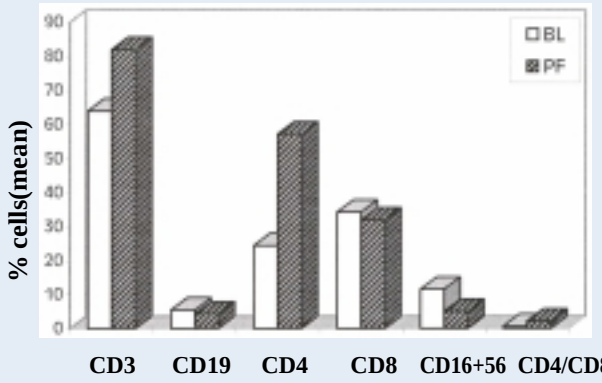
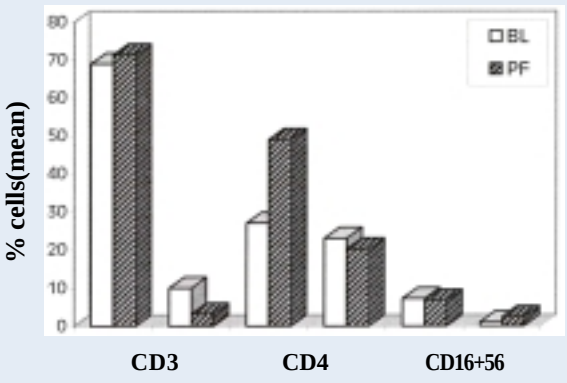


Fig. 2 Cell subset profile in Blood and Pleural Fluid of 5 non TB Pleuritis controls



This increase in the percentage of $CD8^+$ cytotoxic T cells could be compensatory in response to the increased percentage of $CD4^+$ cells, a mechanism to maintain the $CD4^+/CD8^+$ ratio and an additional "acquired" immune activation by these cells to control infection. The increased percentage of $CD4^+$ helper T cells known to be the major IL2 and IFNγ secreting cells, the key cytokines of the Th1 immune response which is said to dominate in the mild disease state and thus denotes a protective immune response.

Immunological consequences of helminth and mycobacterial coinfection

Infection with tissue-invasive and/or intestinal helminths affects a large proportion of human populations living in tropical and subtropical regions.The investigation of the relationship between human helminth infections and the immune response to non-helminth antigens is of public health significance for many reasons. There are several ways in which helminthiases may be a direct or contributive factor in poor immune responses to mycobacteria. If pre-existing infections can influence immune responses against unrelated antigens the implications for the effectiveness of vaccination programs, may be significant.

The present study is designed to determine the effect of intestinal helminth infection (using the presence of any intestinal helminth as the indicator infection) and/or filarial infection on immune responsiveness to mycobacterial antigens. Given the failure of live BCG vaccination in South India in an area in which inhabitants harbor intense geohelminth infections, and in whom 10-20% have active filarial infection as well, this study is of potentially great public health significance

The objectives of the study are to determine the

i. co-prevalence of infection with intestinal helminths and/or lymphatic filariasis and infection with *Mycobacterium tuberculosis.*

ii. effect of intestinal helminth infection or filarial infection on immune responsiveness to mycobacterial antigens

There will be two parts to the study. The first will be a community-based assessment of all individuals to assess directly the co-prevalence of filarial infection and/or intestinal helminth infection with a DTH-type immune response to mycobacterial antigen and with clinical tuberculosis. Co-prevalence or co-infection will be defined as infection with both intestinal helminths and/or filarial parasites and a positive DTH response

to purified protein derivative (PPD). Once baseline data are obtained, all those who are PPD negative (but who harbor helminth parasites) will be randomized to receive either anthelminthics or placebo twice over a six week period. One and 4 months following the last dose, change in PPD status will be assessed as well the intestinal and filarial parasite burden. By assessing these changes, the influence of intestinal helminth or filarial infection on the cellular immune response to *Mycobacterium tuberculosis* can be determined.

The first phase of the study has been completed and the results are being analysed.

Studies on dual signal hypothesis of apoptosis

a. Cell proliferation and Apoptosis in tuberculosis:

Cell proliferation and cell death are two pathways of regulation of the immune response against any antigen that the immune system encounters. By contrast activation induced negative selection is when the immune system eliminates harmful self reactive lymphocytes. A better understanding of these processes provides an insight into the regulation of the immune system.

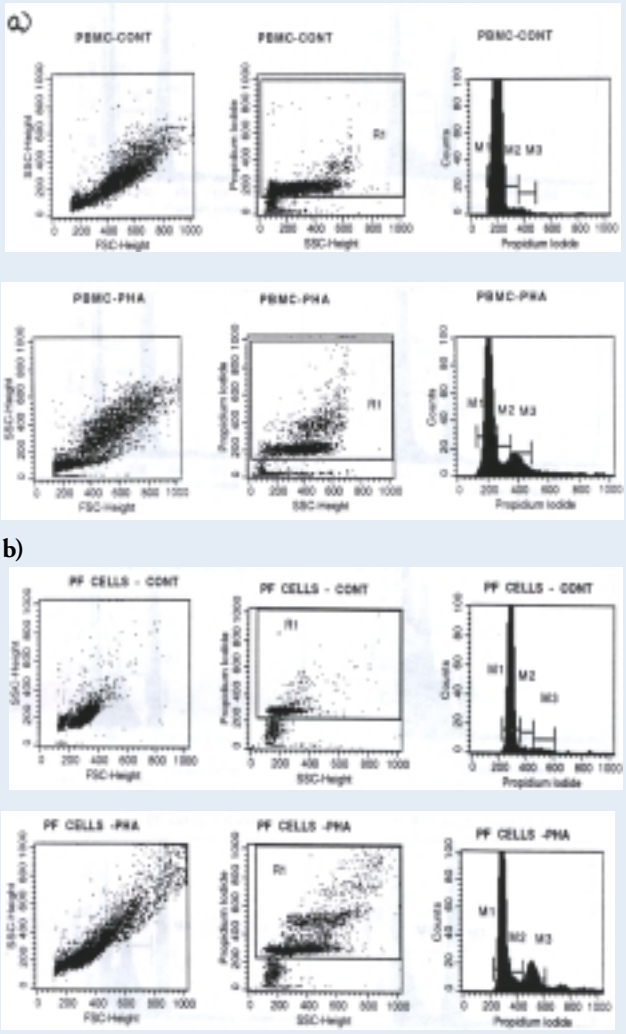
We compared *in vitro* cell proliferation of PBMCs by LTT and cell cycle analysis in normals and tuberculosis patients. Apoptosis was induced by glucocorticoids and anti-CD3 in these cells. The DNA content histogram and cell cycle analysis by flowcytometry was used for studying cell proliferation and detection of apoptosis.

The cell cycle analysis for assaying proliferation of T cells stimulated with PHA showed distinct G2/M peaks in all subjects showing increase in proliferation (Fig.). The distinct phases of cell cycle like G0/G1, S phase and G2/M were assessed by FACS & MODFIT analysis.

Dexamethazone (DM) and anti-CD3 induced apoptosis in PBMCs and the degree of Apoptosis increased with prolonged treatment. Both inducers had a comparatively more significant effect on TB patient samples (PBMCs and PF cells). These preliminary data indicate 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.

Further work is in progress in this direction.

Fig. Representative data of (a) normal PBMCs & (b) PF cells acquired on flow cytometer



The plot of FSC vs SSC gives the general cell characteristics (size and granularity). The second plot gives the amount of propidium iodide staining in cells. The DNA histogram represents GO/GI peak (M1), S phase (M2) and G2/M peak (M3).

b. *In-vivo* study of apoptosis using mycobacterial antigens:

Proliferating cells undergo programmed cell death (PCD)/apoptosis after fixed number of cell divisions. Under physiological conditions mitogens and antigens trigger mitosis and apoptosis simultaneously. Such activation induced apoptosis helps to maintain cellular homeostasis in immune system and determines susceptibility or resistance to pathogenic diseases like tuberculosis.

In the present study, we tried to test dual signal hypothesis under *in vivo* conditions in murine model using PPD as mycobacterial antigen. We studied activation induced apoptosis and proliferation in primary & secondary lymphoid organs and blood.

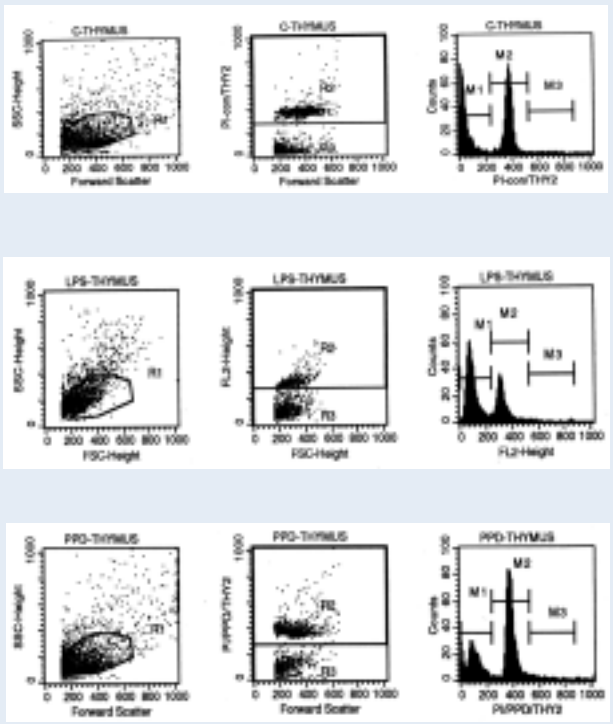
DNA fragmentation assay showed LPS induced apoptosis in thymus and bone marrow, and PPD induced apoptosis in thymus and blood. This showed the hyper responsiveness of immature thymocytes to apoptotic trigger.

FACS analysis (Fig.) shows the size and granularity of mononuclear cell population (RI) in FSC vs SSC plot. Gated analysis revealed proliferating cells (R2) and apoptotic cells (R3). DNA histogram analysis showed hypoploidy peak (MI), G0/G1 peak (M2) and S/G2/M peak (M3) in all the 3 groups.

Our FACS and IPA results showed a basal level of apoptosis and proliferation in all the tissues. The basal level was higher in thymus when compared to other organs. Thus high dosage of antigen and mitogen induced apoptosis and inhibited proliferation to varying degree in different tissues.

Further investigations in this direction are in progress.

Fig. Representative of data of Thymus acquired on Flowcytometer



The plot of FSC vs SSC gives the general cell characteristics (size & granularity). The second plot gives the amount of propidium iodide staining (FL2) cells. The DNA content histogram representing Hypopoidy peak (M1), G_0/G_1 peak (M2) and S/G_2M, peak (M3) is depicted.

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.
D.Sulochana, Ph.D.
S.Ramanujam, B.Sc.,DMLT
S.K.Vasan, Ph.D.
S.Selvakumar, M.Sc.

Awards

XXVI Annual Conference & Symposium on 'Cancer Immunology in the New Millennium', organised by the Indian Immunology Society held at Cancer Research Institute, Mumbai during Jan. 2000. - "HLA-A, B, Tumor necrosis factor–β (TNF-β) and Interleukin-1 receptor antagonist (IL-1RA) gene polymorphism in spinal tuberculosis (extrapulmonary form of tuberculosis)." - **Mr.S.M. Kurian, Dr.P.Selvaraj, Dr.A.M.Reetha, Mrs.N.Charles & Dr.P.R. Narayanan**

"Cell proliferation and Apoptosis in Tuberculosis" - **Ms.Deepa Subramanian & Dr.D. Sulochana**

International Symposium on 'Recent advances in Molecular biology, allergy and Immunology' organized by the M.S. University of Baroda and State University of New York at Buffalo in Association with Indian Academy of Allergy held at Vadodara during Sept. 2000 - "I*n vivo* study on Dual signal hypothesis of Apoptosis using mycobacterial antigens" - **Dr. D. Sulochana &Mr.V.Aravindan**

"R.C. Garg Memorial Award" for the best article published in the Indian Journal of Tuberculosis in 1999 -"Antibody and Lymphocyte responses to *Mycobacterium tuberculosis* culture filtrate antigens in active and quiescent (cured) pulmonary tuberculosis - **H.Uma, P.Selvaraj, A.M.Reetha, Theresa Xavier, R.Prabhakar & P.R.Narayanan**

Capacity Building

Doctor of Philosophy

Dr.S.K.Vasan - Isolation and Characterization of mycobacterial promoters: Cloning and molecular studies of the *gua*A gene encoding GMP synthetase from *Mycobacterium tuberculosis* - Nov. 1999 - The Tamil Nadu Dr.MGR Medical University.

Dr.S.Arvind Pradeep - Cloning, Sequencing and Expression studies of the *ace*A gene encoding Isocitrate Lyase from *Mycobacterium tuberculosis*- Jan. 2000 - The Tamil Nadu Dr.MGR Medical University.

Dr.Nirmala Raman - Role of Natural Killer Cells in Tuberculosis - Mar. 2000 - The Tamil Nadu Dr.MGR Medical University.

Thesis in progress

Ms.K.R.Uma Devi - Purification and evaluation of 38, 30 and 16kDa antigens of *M. tuberculosis* for rapid diagnosis - The Tamil Nadu Dr.M.G.R. Medical University.

Mr.Sunil Mathan Kurian - Studies on gene polymorphisms (HLA & non-HLA) and immune response in spinal tuberculosis - The Tamil Nadu Dr.M.G.R. Medical University.

Mrs.P.Vijayalakshmi - Early detection of *Mycobacterium tuberculosis* - University of Madras

Mr.S.Selvakumar - Gene Regulation in Mycobacteria - The Tamil Nadu Dr.M.G.R. Medical University.

Mr.B.Ramalingam - Immunological and physicochemical characterization of 27kDa protein of *M.tuberculosis* - The Tamil Nadu Dr.M.G.R. Medical University.

Mr.V.Kamalakannan - Cloning, characterization and regulation studies of *guaA* promoter of *Mycobacterium tuberculosis* - University of Madras.

Ms.G.Chandra - Non-HLA gene polymorphisms and immune response in tuberculosis. - University of Madras.

Participation in Conferences / Workshops / Symposia

International

Meeting of the Task Force on 'Community Directed Treatment of Lymphatic Filariasis and Onchocerciasis' TDR/WHO held at Geneva during Mar.1999 - **Dr.V.Kumaraswami** (Chairman)

Workshop on 'Control and Elimination of lymphatic filariasis' organised by CDC held at Atlanta,USA during July 1999 - **Dr.V.Kumaraswami**

International workshop for Training of Trainers on the 'treatment and prevention of lymphedema in lymphatic filariasis' held at Recife, Brazil during May- June - 2000 - **Dr.V.Kumaraswami**

Meeting of the Task Force on 'Filariasis Intervention Research' held at Les Molunes, France during May & Sept.2000 - **Dr.V.Kumaraswami** (Chairman)

Protocol Development Workshop for Phase II on 'Onchocerciasis advocacy' held at Ota, Nigeria during Oct.2000 - **Dr.V.Kumaraswami** (Facilitator)

National

Protocol Development and Data management Training Workshop on 'Lymphatic Filariasis Control' held at Pune during June1999 - **Dr.V.Kumaraswami** (Facilitator)

Interactive Workshop on 'Recent research advances and filariasis elimination campaign in India' held at Vector Control Research Centre, Pondicherry during July 1999 - **Dr.V.Kumaraswami** (Facilitator)

XVII International Congress of Lymphology held at Chennai during Sept.1999 - **Dr.V.Kumaraswami**

UGC sponsored workshop held at Sri Avinashilignam Home Science College, Coimbatore during Sept.1999 - **Dr.Sujatha Narayanan** (Resource person)

Data analysis and report writing for 'ComDT filariasis' held at Vector Control Research Centre. Pondicherry during Nov.1999 - **Dr.V.Kumaraswami** (Facilitator)

Protocol Development and Clinical demonstration Workshop on 'ADL Studies' held at Alleppey during Nov.1999 - **Dr.V.Kumaraswami** (Facilitator)

Workshop on 'Training needs for the National Filariasis Control Programme' held at New Delhi during Dec. 1999 - **Dr.V.Kumaraswami** (Facilitator)

XXVI Annual Conference and Symposium on 'Cancer Immunology in the New Millennium' organised by the Indian Immunology Society held at Cancer Research Institute, Mumbai during Jan.2000.
- "Specific and early detection of IgG, A and M antibodies to *M.tuberculosis* 38kDa antigen in pulmonary tuberculosis" - **Ms.K.R.Uma Devi & Dr.Alamelu Raja**
"Isotype specific antibody response to two purified antigens (30&38kDa) in childhood tuberculosis"
- **Mr.B.Ramalingam, Ms.U.Durga, Dr.Alamelu Raja & Dr.S.Swaminathan**
"Influence of mannose binding lectin and vitamin D receptor gene variants on mantoux status and lymphocyte response in pulmonary tuberculosis"
- **Dr.P. Selvaraj, Dr.A.M. Reetha, Dr.H.Uma & Dr.P.R. Narayanan**
"Immune responses in tuberculous pleuritis"
- **Ms.Kripa, V.Jalapathy & Dr.D.Sulochana**

Workshop Symposium on 'PCR applications in the diagnosis of Infectious Diseases' held at Dr.ALM PGIBMS during Feb.2000 - "Role of PCR & Molecular methods in the diagnosis of tuberculosis"
- **Dr.Sujatha Narayanan**

National Workshop on 'Molecular Diagnostics' organized by Dept. of Biochemistry, University of Kerala held at Tiruvananthapuram during Mar. 2000 – "Molecular Diagnosis of tuberculosis"
- **Dr.Sujatha Narayanan**

Symposium on 'Current status of tuberculosis in India - 2000' organised by Sir Dorabji Tata Centre for Research in Tropical Diseases held at Indian Institute of Science, Bangalore during Mar.2000 - "HLA and non-HLA gene polymorphism in tuberculosis"
- **Dr.P.Selvaraj**

International Symposium on 'Recent advances in Molecular Biology, Allergy and Immunology' held at Baroda during Sept.2000 - **"**Role of non-HLA gene polymorphisms in Susceptibility / Resistance to pulmonary tuberculosis" - **Dr.P.Selvaraj**

International Symposium on 'Molecular Epidemiology and evolutionary genetics of infectious diseases' held at Hyderabad during Nov.2000 - "Molecular Epidemiology" - **Dr.Sujatha Narayanan**

Indo-French workshop on 'Tuberculosis' held at Chennai during Dec.2000 - "Molecular Fingerprinting of *M.tuberculosis* strains to assess the Epidemiological impact of DOTS in a rural setting in South India" **-Dr.Sujatha Narayanan**

"HLA and non-HLA gene polymorphisms in genetic susceptibility/resistance to tuberculosis"- **Dr.P.Selvaraj**

"Improved diagnosis of pulmonary tuberculosis by detection of free and immune complex bound anti-38, 30 and16 kDa antibodies" - **Dr.Alamelu Raja**

Membership in Expert Committees & Special Assignments

Chairman, Task Force on Community Directed Treatment of Lymphatic Filariasis and Onchocerciasis, TDR/WHO, 1999 - **Dr.V.Kumaraswami**

Chairman, Filariasis Intervention Research Task Force, TDR/WHO. 2000 - **Dr.V.Kumaraswami**

Member, Panel of experts on Filariasis, CTD, WHO, Geneva - **Dr.V.Kumaraswami**

Member, Expert Advisory Panel – Filariasis – SEARO - **Dr.V.Kumaraswami**

Member, ICMR Task Force on Filariasis - **Dr.V.Kumaraswami**

Member, Technical Advisory Group on Filariasis, Ministry of Health and Family Welfare, Govt. of India - **Dr.V.Kumaraswami**

Member, State Level Technical Committee for Filariasis Control, Govt.of Tamil Nadu - **Dr.V.Kumaraswami**

Special Invitee, Scientific Advisory Committee, VCRC - **Dr.V.Kumaraswami**

President, Lymphology Society of India - **Dr.V.Kumaraswami**

Member, Editorial Board, Tropical Medicine and International Health - **Dr.V.Kumaraswami**

Member of the Institutional Animal Ethical committee, Madras Veterinary College, Chennai - **Dr.Sujatha Narayanan**

Member of organising committee, Science City, Chennai - **Dr.Sujatha Narayanan**

Member, National Council, Indian Society for Histocompatibility and Immunogenetics - **Dr.P.Selvaraj**

Training Programmes

Training in ELISA, HLA typing, Immuno blotting, techniques in cellular immunology, FACS analysis, PCR and Gene Cloning has been offered to staff and students of neighbouring institutions.

Contact Persons

Immunology & Chemotheraphy of Lymphatic Filariasis
Tuberculosis & helminth co-infection
Geographical information system
Dr.V.Kumaraswami
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Immunodiagnosis of tuberculosis
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Molecular Biology of *M.tuberculosis*
Molecular Epidemiology of tuberculosis
Dr.Sujatha Narayanan
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Immunogenetics of tuberculosis
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Cellular Immunology
Molecular Epidemiology of tuberculosis
Dr.D.Sulochana
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PATHOLOGY

OVERVIEW

The research activity of this Department is mainly concerned with understanding the pathogenetic mechanisms underlying tuberculosis. Clinical material obtained from tuberculous lesions of the lymph node and skin and tissue from an animal model using the guinea pig have been the main sources of study towards this goal. A model for understanding the fibrotic processes following tuberculosis was developed earlier. In addition, the use of third generation staining techniques using enzyme immuno histochemistry to delineate more than 20 host and bacterial products on about 5000 samples has provided valuable insights into some of the pathogenetic mechanisms. These include a) defining the parameters for detecting *M.tuberculosis* antigen(s) in tissue, b) identifying some of the humoral immune mechanisms in the causation of necrosis in tuberculosis and c) describing for the first time, a three week reaction and some of its cellular and molecular correlates elicited by purified protein derivative.

NEW INITIATIVE

Vaccine development
(Collaborative Project with University of Delhi - South Campus)

RESEARCH FOCUS

Cutaneous tuberculosis
Complement system in *Mycobacterium tuberculosis*
Immunomodulatory effect of immune complexes

In situ characterization of the three week reaction evoked by PPD in patients with cutaneous tuberculosis

In order to understand the nature of host immune response to *M.tuberculosis*, we had earlier studied the kinetics of the reaction to a dermal injection of Purified Protein Derivative in patients with cutaneous tuberculosis and histologically characterized the nature of the reaction, 3 and 21days after injection. We found that the PPD-induced reaction persisted upto 3 weeks in a proportion of these patients and histologically this reaction was characterized by the presence of epithelioid cells and a peripheral ring of CD8 positive lymphocytes. The main aims of the present study were to delineate cell markers defined by cluster of differentiation [CD] antigens CD 35, 43 & 68 and expression of cytokines IFN-γ and TGF-β and matrix molecules fibronectin and collagen IV in the PPD-induced granuloma.

Paraffin embedded sections from the biopsy site given PPD,3 and 21 days after injection, were immunohistochemically stained with antibodies against the respective CD antigens, cytokines and matrix molecules.

CD 68 & IFN-γ positive staining are shown in figures A & B respectively.

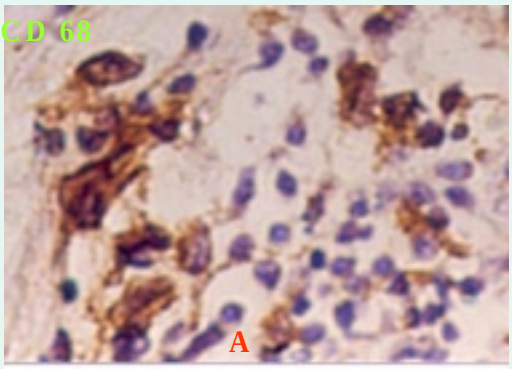


Fig.A. CD 68 positive cells stained dark brown; contrast this with unstained lymphocytes (blue)

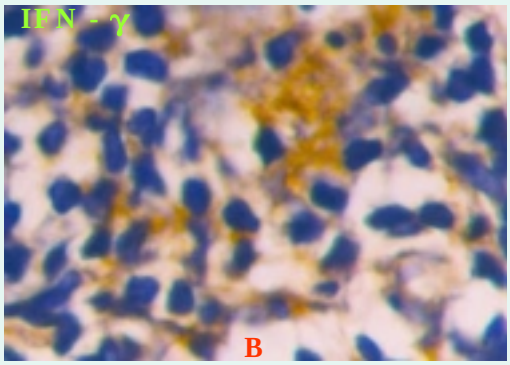


Fig.B. – IFN γ positive cells stained yellowish brown; note the absence of IFNγ in many other cells.

The levels of the different markers obtained on days 3 & 21 are shown in the following table:

Markers	Day 3	Day 21
CD 35	6.2±1.8	4.5±0.7
CD 68	37.1±21.4#	58.9±17.3
CD 43	68.6±11.8	67.1±17.5
IFN-γ	45.0±20.2	39.0±23.4
TGF-β	30.7±6.7~	15.7±6.5
FN	17.1±7.0*	10.2±3.2

(Values shown as Mean ± SD of the % of positively stained cells. # p <0.05: ~ p <0.001: * p <0.05)

Staining for collagen IV not quantitated as it was seen mainly in the matrix.

The significant findings of this study are as follows:

- **CD68 positive cells more on day 21 than on day 3, while TGF-β less on day 21.**
- **Outcome of the granulomatous process may depend on the resultant interaction between these two cytokines.**
- **Collagen and fibronectin seen from day 3 indicate that fibrogenic process in tuberculosis is initiated from an early stage of granuloma formation.**
- **Resolution of the granuloma was indicated by less fibronectin on day 21.**

The prognostic significance of this three week reaction needs to be evaluated in prospective studies of patients with tuberculosis and their contacts.

Interaction between the complement system and *Mycobacterium tuberculosis*

It has previously been shown that a number of mycobcateria including *M.tuberculosis* and some of their components have the capacity to activate the complement system through both the classical and the alternative pathways. It has further been shown that the initial activation and coating of the bacilli with complement components can have consequences affecting the subsequent host response to the organism. However, this has not been studied in regard to variations in the strain of the tubercle bacillus and the effect of antibody on this interaction. Hence a study was planned to look at the

i. activation profile of standard (H_{37} Rv & H_{37} Ra) strains and isolates of *M.tuberculosis* from patients.

ii. effect of antimycobacterial antibodies on activation of complement pathways.

iii. role of cytokines and complement during apoptosis of *M.tuberculosis*.

An ELISA method to detect solid phase activation of complement system on the surface of *M.tuberculosis* has been standardized. The kinetics and the mechanics of the binding of various subcomponents of C3 are being studied using a number of antibodies.

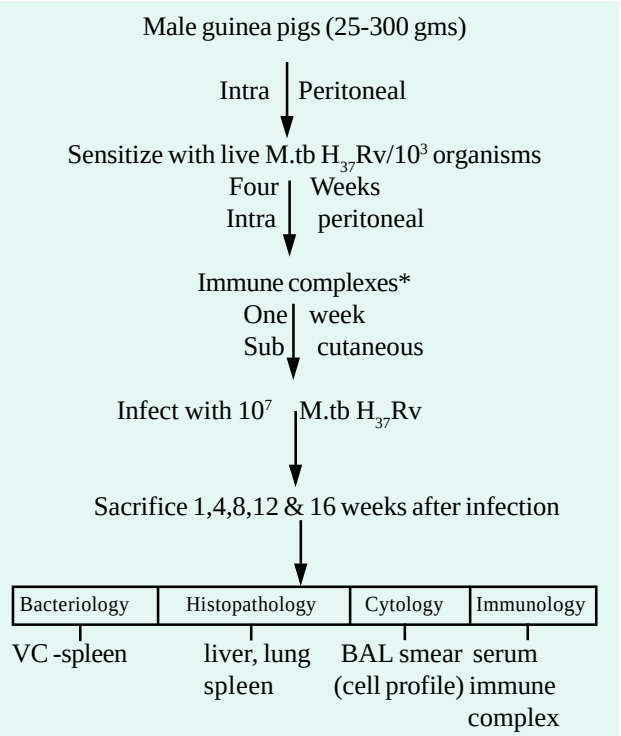
Immunomodulatory effect of immune complexes in a guinea pig model for post primary tuberculosis

Post primary form of tuberculosis is by far the commonest type encountered in India. Due to repeated exposure to environmental mycobacteria as well as *M.tuberculosis*, contacts of patients with tuberculosis have high amounts of circulating immune complexes. Antigen-antibody complexes can modulate the subsequent immune response to a given antigen. We are evaluating the immunomodulatory role of preformed immune complexes containing *M.tuberculosis* and antibody in various proportions in a guinea pig model for post primary tuberculosis.

The methodology involves presensitising guinea pigs with tubercle bacilli and immune complexes followed by infecting them with *M.tuberculosis* H_{37}Rv and determining viable counts of the bacilli in various organs at different time points.

Antibody to *M.tuberculosis* was raised in rabbits, purified from serum using ammonium sulphate and the titre of the antibody was checked using ELISA. Antigen-antibody complexes in antigen excess and antibody excess were prepared on a volume/volume basis at a proportion of 49:1.

The methodology used is given in the following flow diagram:



*Equivalent amounts of antigen alone or antibody alone appropriately diluted in normal saline or normal saline alone are injected into groups of guinea pigs.

Staff List

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

Capacity Building

One month training course on "MS Office"during Sept.-Oct.,1999 -**Mr.S.Nambirajan**

Participation in Conferences / Workshops / Symposia

National

Symposium on Collagen held at Central Leather Research Institute, Chennai during Jan.1999 - **Dr.V.D.Ramanathan**

National Science Day Organized by the Dept. of Bitotechnology, Centre for Biotechnology, Anna University held at Chennai during Feb.1999 -"A Bird's Eye view of Tuberculosis" - **Dr.V.D.Ramanathan**

National Environmental Awareness Campaign 1998-99 on 'Water resources for the next millennium' organised by Dept. of Environment, Govt. of India held at Dept. of Zoology, Loyola College, Chennai during Mar.1999 - "Water borne diseases" - **Dr.V.D.Ramanathan**

Workshop on 'Simulation Model for Leprosy Transmission Control' organized by WHO-ICMR held at National Institute of Epidemiology, Chennai during Apr.1999
- **Dr.V.D.Ramanathan**

ICMR/WHO National Workshop on 'Health Research Management', held at Tuberculosis Research Centre, Chennai during Sept.1999 - **Dr.V.D.Ramanathan**

WHO/IUIS Refresher Course on 'Immunology, Vaccinology & Biotechnology applied to infectious disease' held at Pune during Dec.1999 -"Physiopathology of tuberculosis" - **Dr.V.D.Ramanathan**

5th International Conference on 'Emerging Infectious Diseases in the Pacific Rim' held at Chennai during Jan. 2000 - **Dr.V.D.Ramanathan**

Symposium on "Microhorizon" Sponsored and Organized by the Dept. of Microbiology, Jaya College of Arts & Sciences, Chennai during Feb.2000 -"The immunopathology of tuberculosis" - **Dr.V.D.Ramanathan**

International

Pre Nobel Symposium held at Karolinska Institute, Stockholm, Sweden during Aug.2000 - "Pathology of tuberculosis with special reference to pathogenesis"
- **Dr.V.D.Ramanathan**

Nobel Symposium on 'Prevention and Treatment of Tuberculosis in the coming Century' held at Karolinska Institute, Stockholm, Sweden during Aug.2000 - "Immunohistological characterisation of the three week Mantoux reaction in cutaneous tuberculosis patients"
- **Dr.V.D.Ramanathan**

Membership in Expert Committees & Special Assignments

Consultant histopathologist – CLTRI, Chengalput.
- **Dr.V.D.Ramanathan**

Member, Advisory Committee on 'ILEP Nerve Function Impairment and Reaction Study' -**Dr.V.D.Ramanathan**

Training Programmes

Training on immunohistological staining was given to medical & paramedical students from neighbouring institutions.

Contact Person

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

BIOCHEMISTRY

OVERVIEW

This Department offers biochemical support to the controlled clinical trials carried at our centre. In the past, extensive work was carried out to determine acetylator phenotyping using blood, urine and saliva. The mechanism of development of toxicity to rifampicin, isoniazid and pyrazinamide has been investigated. A number of pharmacokinetic studies with different combinations of anti-tuberculosis drugs have been undertaken. Simple methods for estimation of anti-tuberculosis drugs in body fluids have been standardised and it was established that non-invasive method of estimating salivary levels of drugs could replace plasma. Recently the department has undertaken bioavailability studies of fixed dose combinations of anti-tuberculosis drugs. Monitoring of drug toxicity in tuberculous patients undergoing treatment at the centre and assay of anti-tuberculosis drugs in pharmaceutical preparations to check for their quality form the routine activities of the department.

NEW INITIATIVE

Clinical Pharmacology Unit
Traditional plants and drugs in tuberculosis

RESEARCH FOCUS

Pharmacokinetics of ofloxacin
Bioavailability of rifampicin (standardisation of rifampicin by HPLC)

Pharmacokinetics of ofloxacin, rifampicin, isoniazid and pyrazinamide when administered alone and in combination

Ofloxacin (O) has proved to be a valuable addition to the available anti-TB drugs. Since limited information is currently available on the pharmacokinetic interactions of O with other anti-TB drugs, viz., rifampicin (R), isoniazid (H) and pyrazinamide (Z), a study was undertaken to investigate the bioavailability of O when given alone and in combination with the above mentioned drugs in healthy subjects.

Twelve male healthy volunteers were randomly allocated to the different groups, namely, O alone, O+R,O+H,O+Z and O+R+H+Z. Blood and saliva samples were collected at 1,2,3,6 and 8 hours after drug administration and total urine excreted over the periods 0-4 and 4-8 hours was also collected. Plasma concentrations of all the four drugs, salivary concentrations of O and urinary excretion of O, total R, H and its metabolites (acetyl H and isonicotinic acid) and Z and pyrazinoic acid were determined after coding the samples. The pharmacokinetic variables such as peak concentration (C-max), the time taken to attain the peak concentration (t-max), the area under the time-concentration curve (AUC) and half-life (t½) were calculated from the plasma and salivary concentrations. Further, the proportion (%) of the doses excreted as unchanged drugs and their primary metabolites in urine collected over the periods 0-4 and 0-8 hours were also calculated.

The results of this study indicate that the pharmacokinetic properties of O are not modified when administered with other anti-TB drugs viz., R, H and Z and also the bioavailability of these drugs do not get altered when given along with O.

In continuation of this study, a simple and rapid high performance liquid chromatography method for determination of O in plasma and urine was standardised. Monitoring of O concentrations in body fluids may be valuable to adjust the drug dosage and to study drug-drug interactions when co-administered with other anti-tuberculosis drugs.

The HPLC method involved deproteinisation of plasma with perchloric acid and analysis of the supernatant using reverse phase C18 column and fluorescence detection at an excitation wavelength of 290 nm and an emission wavelength of 460 nm. The assay was linear from 0.5 to 10.0 µg/ml of O. The coefficient of variation of intra- and inter-day assays were lower than 5%. The average recovery of O from plasma was 93 %.

The above method was applied to 60 plasma and 24 urine samples (stored at –20°C) obtained from 12 healthy volunteers as described previously. These samples were previously analysed for O concentrations by microbiological assay (MBA) using *Escherichia coli* as test organism.

Figure 1 represents the comparison of plasma concentrations determined by HPLC and MBA method. It can be seen that majority of data points fall away from the line of regression indicating poor agreement between the two methods (r=0.76). These results also suggest that MBA underestimates the microbiological activity of O in the sample. Comparison of the urinary data showed better agreement (r = 0.99) (Fig.2).

Fig.1. Plasma concentrations of O (µg/ml) by two methods

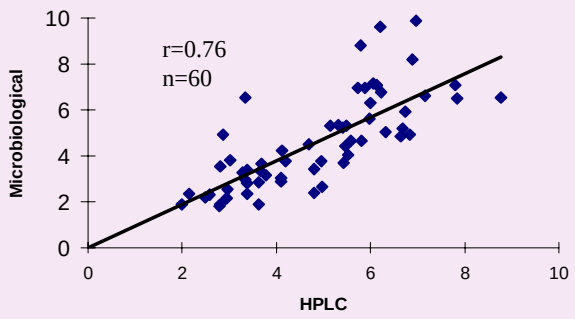
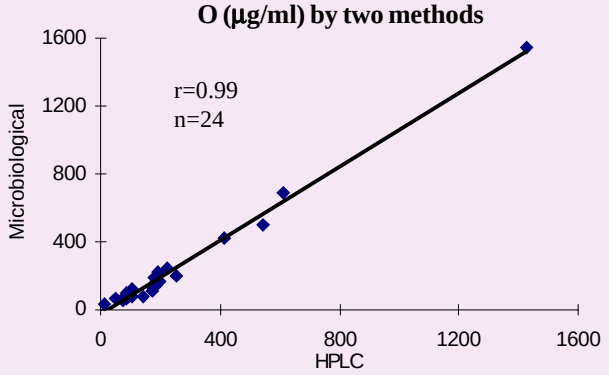


Fig.2. Urinary concentrations of O (µg/ml) by two methods



On the basis of the O concentrations measured in blood and urine by both the methods, the summary measures of O, namely, AUC (0-8hr) and percentage dose excreted of the drug excreted in urine were calculated. The mean values of AUC were 30.7 and 28.1 µg/ml/hrs. by HPLC and MBA respectively. The proportion of doses excreted were 39% and 38% by HPLC and MBA respectively. Neither of the differences were statistically significant ($p > 0.2$).

The HPLC method developed for determination of O in plasma and urine is simple, sensitive and precise with an accuracy of greater than 90 %. The assay is neither laborious nor time consuming than the bioassay and permits processing of more samples and therefore, more suitable for kinetic studies.

Standardisation of HPLC method for estimation of rifampicin in plasma

During this year, a HPLC method for determination of rifampicin(R) concentrations in plasma was standardised.

In earlier studies, R levels in plasma were estimated employing the microbiological assay. Although this technique is simple, the major disadvantage of the method is that it measures both R and its metabolite, desacetyl R(DesR) as total R. Hence the necessity to standardise a HPLC method which could differentially estimate R and DesR occurred.

This method involves protein precipitation of plasma with acetonitrile, concentrating the content by vacuum drying and reconstituting with mobile phase followed by reverse phase liquid chromatography using a RP_{18} column. The mobile phase consisted of acetonitrile : 0.05M phosphate buffer (45:55) and the internal standard was rifapentine. The retention times for DesR, R and rifapentine were 2,3 and 6 minutes respectively. The assay was linear from 0.7 to 14.0 µg/ml of R. Precision of the assay ranged from 4.9 to 7.9 % (within day) and 4.5 to 7.1% (between day). The mean recovery of added R concentrations was 97 %. The method was specific and none of the other anti-tuberculosis drugs interfered in the estimation.

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.
Vijayalakshmi Sreedhar, M.Sc.
K.Silambhuchelvi, M.Sc.,
A.K.Hemanth Kumar, M.Sc.,DMT.

Awards

International Symposium on 'Luminescence and its applications' held at M.S. University, Baroda during Feb.2000 -"Biomedical application of luminescence: Luciferase reporter phage assay for rapid drug susceptibility testing of *Mycobacterium tuberculosis* isolates" - **Dr.K.Jayashankar**

Capacity Building

Doctor of Philosophy

Dr.Geetha Ramachandran - Studies of Cytochrome P-450 in relation to drug resistance in mycobacteria – Nov.1999 - The Tamil Nadu Dr. MGR Medical University.

Thesis in progress

Mr.A.K.Hemanth Kumar – Biochemical and pharmacological aspects of ofloxacin in tuberculosis -The Tamil Nadu Dr.MGR Medical University

Ms.Aruna - Biochemical aspects of mycobacterial infection - The Tamil Nadu Dr.MGR Medical University

Ms.R.Gayathri - Biochemical aspects of mycobacterial infection -The Tamil Nadu Dr.MGR Medical University

Seminar on 'Developing HPLC separation' conducted by WATERS (India) held at Chennai during May 1999 **- Mrs.Chandra Immanuel & Mr.A.K.Hemanth Kumar**

"Recent trends in chromatography" conducted by SPINCO Biotech. Pvt. Ltd. held at Chennai during July 1999 - **Mr.A.K.Hemanth Kumar**

Seminar on "New developments in chromatography, water and waste-water analysis" conducted by Associated Instruments and Chemicals held at Chennai during Aug. 2000 - **Dr.Prema Gurumurthy, Mrs.Chandra Immanuel, Mrs.Lalitha Victor & Mr.A.K.Hemanth Kumar**

Seminar on "Frontiers in Chromatography" conducted by Chromatographic Society of India held at Chennai during Sept.2000 - **Dr.Prema Gurumurthy, Mrs.Lalitha Victor & Mr.A.K.Hemanth Kumar**

Participation in Conferences / Workshops / Symposia

National

UGC sponsored State level seminar on 'Recent Trends in Biochemistry' held at PSG College of Arts and Science Coimbatore during Feb.1999 - "Biochemical aspects of adverse reactions to antituberculosis drugs**"**
- Dr.Prema Gurumurthy

International symposium on 'Transcription Assembly and nucleic acid – protein interactions' held at Indian Institute of Science, Bangalore during June 1999
- Dr. Prema Gurumurthy

Satellite Symposium on "Free Medicals in Health and Diseases" held at Dr.ALMPGIBMS, Chennai during Dec. 1999 - "The generation of reactive oxygen intermediates during infection with different strains of mycobacteria"
- Dr.M.Kannapiran

54th National Conference on TB & Chest Diseases held at Patna during Dec.1999 - "Mechanism of drug resistance in *M.tuberculosis* with reference to Cytochrome P-450"
- Dr.Geetha Ramachandran

Profiles in Medical Laboratory technology held at Loyola Institute of Vocational Education (LIVE), Loyola College, Chennai during Feb.2000 - "Pharmacokinetic drug interactions in the chemotherapy of tuberculosis"
- Dr.Prema Gurumurthy

Membership in Expert Committees & Special Assignments

Member of organising committee, Science City, Chennai
- Dr.Prema Gurumurthy

Training Programmes

Training in clinical biochemistry & pharmacokinetics was offered to many students from neighbouring institutions.

Contact Persons

Bioavailability of Fixed Dose Combinations
Pharmacokinetics of anti-tuberculosis drugs
Assays of anti-tuberculosis drugs
Clinical biochemistry
Dr.Prema Gurumurthy
trcicmr@md3.vsnl.net.in(biochemistry)

Traditional plants & drugs
Trace element Metabolism
Dr.M.Kannapiran
trcicmr@md3.vsnl.net.in(biochemistry)

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

STATISTICS

OVERVIEW

The Statistics department offers support in designing investigations which include the types of controls to be employed, the number of patients/specimens to be studied etc. Randomization techniques are made use of in allocating patients to various regimens prescribed and also in constructing different groups of patients. The department assists in evolving an all-inclusive protocol of various investigations and procedures for the successful conduct of controlled clinical trials both during treatment and follow up. Large scale data on various clinical and laboratory investigations are organized after systematic scrutiny for accuracy of information and one to one identification with the subject concerned and comprehensive documentation on planned formats like analysis and treatment cards. The department organizes drug assays to keep a systematic watch on the quality of drugs supplied by the pharmaceuticals. It undertakes randomization procedure for coding of laboratory specimens to eliminate bias in the various tests performed in addition to subsequent decoding and analyses. Monitoring of laboratory standards through quality control techniques is another highlight of this department.

NEW INITIATIVES

Conduct of National health surveys, namely, coverage Evaluation survey of Universal Immunization Programme
Survey on usage and acceptabilities of Indian System of Medicine and Homeopathy

RESEARCH FOCUS

Basic and applied statistical methodologies
Survival analysis, non linear regressions
Risk analysis, artificial neural networks
Health economics
HIV/AIDS projections

Staff List

Fathima Rahman, B.Sc., Stat. Dip.(ISI)
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).
D.Rajappa, B.Sc., Dip in Stat.
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, M.Sc.
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.

Capacity Building

Mr.V.Chandrasekaran & Dr. D.Vijaya Bhaskara Rao - RNTCP training for " Senior Treatment Supervisor" (STS) held at TRC, Chennai during Feb.1999

Mr.M.Duraipandian, Mr.V.Sundaram & Mr.V.Chandrasekharan - Training course on Visual Basic 6.0 held at Centre for Reliability, Chennai during July 1999

Mr.B.Vaidyanathan, Mr.D.Suryanarayanan & Mr.C.Ponnuraja - Training course on Oracle & Developer 2000 held at Centre for Reliability, Chennai during July 1999

Mr.L.Sekar, Mr.C.Ponnuraja & Mr.Ashok Naik Chandu - Training course on Oracle 8.0 & Visual Basic 6.0 held at Stenographers's Guild,Chennai during Oct.1999

Mr.M.Subhash Chandra Bose & Mr.L.Sekar - RNTCP training for "Senior Treatment Supervisor" (STS) held at TRC, Chennai during Jan.2000

Mr.Naik Ashok Chandu & Mr.C.Ponnuraja - RNTCP training for " Senior Treatment Supervisor" (STS) held at TRC, Chennai during Feb.2000

Participation in Conferences / Workshops / Symposia

Workshop on "Simulation Model for Leprosy Transmission and Control" held at the Institute for Research in Medical statistics, Chennai during Apr.1999 - **Dr.P.Venkatesan**

Technical meeting on "Operational issues in the National Sample Survey to estimate the ARI of Tuberculosis in India" held at National Tuberculosis Institute, Bangalore during May 1999 - **Dr.P.Venkatesan**

7th Annual Tamil Nadu Conference on TB and Chest Diseases held at Nagercoil during May 1999
- "Demographic profile of treatment compliance in tuberculosis patients admitted to controlled clinical trials" - **Mr.L.Sekar**
- "Summarising results of a controlled clinical trial using NNT approach" - **Mr.M.Subhash Chandra Bose**
- "The influence of gender on action taking behaviour among sputum positive pulmonary tuberculosis patients" - **Mr.V.Chandrasekaran**
- "Caution deposit for compliance:A case study in West Godavari" - **Dr.D.Vijaya Bhasakara Rao**

'Refresher Course on Computer Applications' for College teachers conducted by the Academic Staff College, University of Madras held at Chennai during July 1999
- "Recent Advances in Applied Data Analysis"
- **Dr.P.Venkatesan** (Resource Person)

XVI Annual Conference of Indian Society for Medical Statistics and V Biennial Conference of the International Biometric Society held at NIMHANS, Bangalore during

Dec.1999 - (i) "Fundamentals ofSurvival Analysis" (Invited paper) (ii) "Can ANN. Replace Multivariate Statistics in Biomedical Applications?" (iii) "New Approach for Adjusting Correlated Binary data".
- **Dr.P.Venkatesan**

WHO workshop on EPI-INFO and survey Design/ Analysis held at Chennai during Dec.1999
- **Dr.D.Vijaya Bhaskara Rao**

54th National Conference on Tuberculosis and Chest Diseases held at Patna during Dec.1999 - "Impact of tuberculosis on men" - **Mr.D.Suryanarayanan**

Symposium on "Mathematical Methods and Applications" in honour of Srinivas Ramanujan held at I.I.T., Chennai during Dec.1999 - **Dr.P.Venkatesan**

3rd International Conference on "Operational Research and Game Theory (ICORGT2000)" held at I.I.T., Chennai during Jan.2000 - "A Lot Quality Assurance Model for Data Evaluation in Health Information System"
- **Dr.P.Venkatesan**

International Symposium on " Medical Genetics in 2001" held at Sri Ramachandra Medical College and Research Institute, Chennai during Sept.2000 - **Dr.P.Venkatesan**

XVIII Annual Conference of Indian Society for Medical Statistics, held at CJIL, Agra during Sept.2000 - (i) "Methods for Projection of HIV/AIDS Epidemic" (Invited Paper) (ii) "Frailty Models in the Analysis of Survival Data - **Dr.P.Venkatesan**

International Symposium on 'Advances in Statistical Methods and applications' held at Dept. of Statistics University of Madras, Chennai during Dec.2000
- "A Neural Network Model for Survival Data"
- **Dr.P.Venkatesan**

International Symposium on 'Advances in Statistical Methods and applications' held at Dept. of Statistics University of Madras, Chennai during Dec.2000
- "An Application of Summary Statistics to Clinical Trial Data using Piecewise Linear Growth Curve Model" - **Mr.V.Chandrasekaran**

Membership in Expert Committees & Special Assignments

Editor – ISMS Bulletin- An official publication of the Indian Society for Medical Statistics since Jan. 99
- **Dr.P.Venkatesan**

Chief Guest in the Annual Convention of Mathematical Association, Queen Marys College, Chennai during Feb.1999 - "Recent Developments in Operations Research and Their Applications" (invited paper)
- **Dr.P.Venkatesan**

Expert Committee Member – Committee on Mathematics and Statistics Education in the Next Decade for updating syllabi for Mathematics and Statistics formed by the Tamil Nadu State Council for Higher Education during March 99
- **Dr.P.Venkatesan**

Chief Guest in the Annual Conference of Statistical Association, SDNB Vaishnava College, Chennai during Dec.1999 - "Recent Developments in Medical Statistics" - **Dr.P.Venkatesan**

Co-ordinator – Coverage Evaluation Survey for the Universal Immunization Programme - 1999, Dept. of Family Welfare, Ministry of Health and Family Welfare, Govt. of India, in the states of Andhra Pradesh and Karnataka - **Dr.P.Venkatesan**

Co-ordinator – Survey on Usage and Acceptability of Indian System of Medicine and Homeopathy – 2000, Dept. of ISM & H, Ministry of Health and Family Welfare, Govt. of India, in the states of Andhra Pradesh, Karnataka and Tamil Nadu
- **Dr. P.Venkatesan**

Chief Guest in the Annual Conference of the Mathematics and Statistics Association, Sri Kanyaka Parameswari College of Arts and Science for Women, Chennai, during Dec.2000 - "Recent advances in computational Mathematics and Statistics" - **Dr.P.Venkatesan**

Advocacy

STS and SSTS training during Feb.1999 - **Dr.D.Vijaya Bhasakara Rao** (Observer & Facilitator)

Training for Senior Treatment Supervisors (STS)during Mar.1999 -**Mr.V.Chandrasekaran** (Observer)

Training for a staff of District Tuberculosis Centre, West Godavari, Eluru on NTP and preparation of Monthly, Quarterly and Annual Reports during Sept.1999.
- Dr.D.Vijaya Bhasakara Rao

Training Programme in the training programme on Environmental Epidemiology' during Feb.2000
- **Dr.P.Venkatesan** (Trainer/Facilitator)

Six research scholars are currently pursuing their research leading to Ph.D. degree registered under University of Madras in the following topics (I) Artificial Neural Networks (II) Survival Analysis (III) Competing Risks (IV) Reliability (V) Generalized Estimating Equation and (VI) HIV/AIDS Modelling. (Guide - **Dr.P. Venkatesan**).

One student is current pursuing his M.S. (Software System) external degree programme under Birla Institute of Technology and Science, Pilani.
(Guide - **Dr.P.Venkatesan**)

Contact Persons

Mrs.Fathima Rahman
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Dr.P.Venkatesan
trcicmr@md3.vsnl.net.in(bic)

LIBRARY & INFORMATION DIVISION

The Library & Information Division of Tuberculosis Research Centre has a fairly large collection of books and scientific journals on different aspects of tuberculosis and other related subjects. Books are classified according to the Dewey Decimal Classification Scheme (DDC). The library caters to the needs of the scientific community in and around Chennai.

During the period under coverage the Library services were expanded with a number of value added services such as Medline, NUCSSI, UCCDH and an in–house fortnightly publication, namely, "TB ALERT", which comprises of recent articles related to tuberculosis published in journals subscribed by TRC. A database of TRC Publications (TRCPUB) and a bibliographic database on Tuberculosis are being maintained. Library is having a dial-up Internet connectivity with VSNL, where the Library acts as a Central Node for Electronic Mail co-ordination agency for all the staff of the Centre. There are plans of procuring a CD TOWER to offer the MEDLINE: CD-ROM search facility throughout the LAN to the staff of the centre.

Staff List

R.Rathinasabapathi, M.A.,M.L.I.S.
V.Lalithamma

Capacity Building

"Application of Information Technology in Health Science Libraries" organized by the National Medical Library (NML), New Delhi held at National Institute of Mental Health & Neurosciences, Bangalore during Oct.1999 - **Mr.R.Rathinasabapathi**

"Electronic Publishing"conducted by INSDOC held at New Delhi during Sept.2000
- **Mr.R.Rathinasabapathi**

Participation in Conferences / Workshops / Symposia

CALIBER 2000 (Information Services in a Networked Environment in India) jointly organized by the University of Madras, Chennai and INFLIBNET Centre, Ahmedabad during Feb. 2000 - "Internet: a briefing for layman"- **Mr.R.Rathinasabapathi**

Workshop on "Virtual Resource Sharing" conducted by The Tamil Nadu Dr. MGR Medical University held at Chennai during Feb. 2000
- **Mr.R.Rathinasabapathi**

Contact Person

Mr.R.Rathinasabapathi
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ADMINISTRATION & SUPPORT SERVICES

Staff List

V.Lakshminarayanan, B.Com., A.C.S.
M.Subramanian, B.Com.
C.J.Arunachalam,
Venkata Rama Devi
M.R.Srinivasan
Padma Balasubramanian
K.Jayarajan
J.Santhakumari
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

Contact Persons

Mr.M.Subramanian
Sr. Administrative officer
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trcicmr@md3.vsnl.net.in(accnt)

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

Mr.C.J.Arunachalam
Purchase officer
trcicmr@md3.vsnl.net.in(purchase)

LIST OF PUBLICATIONS 1999-2000

International:

1. Gurumurthy, P., Ramachandran, G., Vijayalakshmi, S., Hemanth Kumar, A.K., Venkatesan,P., Chandrasekaran, V., Vijayasekaran, V., Kumaraswami, V., Prabhakar, R. Bioavailability of rifampicin, isoniazid and pyrazinamide in a triple drug formulation: Comparison of plasma and urine kinetics. *International Journal of Tuberculosis and Lung Disease,* 1999, **3**, 119-125.

2. Ramachandran, R., Balasubramanian, R., Muniyandi, M., Shanmugam, G., Xavier, T., Venkatesan, P. Socio-economic impact of tuberculosis on patients and family in India. *International Journal of Tuberculosis and Lung Disease,* 1999, **3**, 869-877.

3. Parthasarathy,R., Sriram,K., Santha,T., Prabhakar,R., Somasundaram,P.R., Sivasubramanian,S. Short-course chemotherapy for tuberculosis of the spine: A comparison between ambulant treatment and radical surgery - a ten year report. *The Journal of Bone and Joint Surgery,* 1999, **81-B**, 464-471.

4. Uma,H., Selvaraj,P., Reetha,A.M., Xavier,T., Prabhakar,R., Narayanan,P.R. Influence of HLA-DR antigens on lymphocyte response to *Mycobacterium tuberculosis* culture filtrate antigens and mitogens in pulmonary tuberculosis. *Tubercle and Lung Disease,* 1999, **79**, 199-206.

5. Selvaraj,P., Narayanan,P.R.,Reetha, A.M. Association of functional mutant homozygotes of the mannose binding protein gene with susceptibility to pulmonary tuberculosis in India. *Tubercle and Lung Disease,* 1999, **79**, 221-227.

6. Ravikumar,M., Dheenadayalan,V., Rajaram,K., Shanmuga Lakshmi,S., Paul Kumaran,P., Paramasivan,C.N., Balakrishnan,K., Pitchappan,R.M. Association of HLA-DRB1, DQB1 and DPB1 alleles with pulmonary tuberculosis in South India. *Tubercle and Lung Disease,* 1999, **79**, 309-317.

7. Riska,P.F., Su,Y., Bardarov,S., Freundlich,L., Sarkis,G., Hatfull,G., Carriere,C., Kumar,V., Chan,J., Jacobs,W.R. Rapid film-based determination of antibiotic susceptibilities of *Mycobacterium tuberculosis* strains by using a Luciferase reporter phage and the Bronx box. *Journal of Clinical Microbiology,* 1999, **37**, 1144-1149.

8. Swaminathan,S., Gong,J., Zhang,M., Samten, B., Hanna,L.E., Narayanan,P.R., Barnes,P.F. Cytokine production in children with tuberculous infection and disease. *Clinical Infectious Diseases,* 1999, **28**, 1290-1293.

9. Hari,L., Suryanarayanan,D., Thomas,A., Joseph,P. An assessment of the value of midfinger smears in multibacillary leprosy patients. *Leprosy Review,* 1999, **70**, 47-51.

10. Shenoy,R.K., Kumaraswami,V., Suma,T.K., Radhakuttyamma,G. A double-blind, placebo-controlled study of the efficacy of oral pencillin diethylcarbamazine or local treatment of the affected limb in preventing acute lymphoedema caused by Brugian filariasis. *Annals of Tropical Medicine & Parasitology,* 1999, **93**, 367-377.

11. Shenoy,R.K., Suma,T.K., Kumaraswami,V. Treatment of microfilaraemia of asymptomatic Brugian filariasis with single dose of ivermectin, diethylcarbamazine or albendazole in various combinations. *Annals of Tropical Medicine & Parasitology* ,1999,**93**, 643-651.

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