Tuberculosis Research Centre
Chennai

Annual Report
2002-2003

Dr. C. V. Ramakrishnan

1921-2003

We dedicate this issue of the TRC Annual Report to the memory of Dr. C. V. Ramakrishnan, one of the founding fathers of the Tuberculosis Research Centre, and a beacon of inspiration for all of us.

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PREFACE

Forty-seven years ago, the Indian Council of Medical Research launched a research centre to provide essential tools for the control of tuberculosis. Looking over the accomplishments of the past year, we can be confident that the Council will be proud of what has become of its initiative. We, at the Tuberculosis Research Centre, have not only continued to do quality clinical, laboratory and epidemiological research that has provided many tools for tuberculosis control but also trained more than 3000 persons (locally, regionally, nationally and internationally) to apply these tools. A retrospective of the past year reveals more than just a list of impressive accomplishments. It reveals a team spirit and guiding purpose behind our work that binds us together and gives the Tuberculosis Research Centre an identity. It is no wonder that we have become one of the nation's major tuberculosis research and resource centres.

The Tuberculosis Research Centre is at an important stage in its development. It is poised to become an international centre of excellence in biomedical research after having carved a niche for itself in the field of chemotherapy of tuberculosis. In order to achieve this status we need to further strengthen our research in clinical and epidemiological sciences and expand research in basic sciences. With the support of the Director General of ICMR, this process has now been streamlined and in the very near future we expect a rapid growth of new talents and approaches in the field of basic sciences.

The Tuberculosis Research Centre is a large and complex institution. Five hundred and eighty staff members located in Chennai, Tiruvallur and Madurai support the vision and mission of the institution. This report is a culmination of hours of discussion, large number of meetings and several drafts and represents the efforts of several staff members to provide you an insight into our triumphs and achievements in this past year. I am grateful to all those who made this report a reality.

We look forward to your critical appraisal of our research activities as well as your suggestions for improvement.

P. R. Narayanan
Director

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DISTINGUISHED VISITORS

"My experience with TRC and other health staff in a weeklong workshop has gone beyond positive to superlative. The atmosphere of warmth, openness, receptiveness, and collaboration makes me want to return again and again."

Prof. Elliott Churchill
Centers for
Disease Control
USA

"I was singularly honored to have had the privilege to visit such a historical site in TB control and to see the remarkable way that it is forging ahead to lead India and the world TB community. BRAVO!"

Dr. Reuben Granich
WHO, SEARO
New Delhi

"As a "young" member of the global TB community it was a great privilege to visit TRC, both to salute your past achievements and look forward to future global relative achievements."

Dr. Fraser Wares,
WHO, SEARO
New Delhi

"Another exciting visit to this great learning centre. My "TB" life was really enriched greatly by my association with TRC."

Dr. Mukund Uplekar
WHO, Geneva

"I am very impressed by the activities of the institute, which I hope in due course of time might evolve new targets for the eradication of the deadly disease."

Dr. C.L. Kaul
Director
NIPER, Chandigarh

"I was very impressed by the workings of the Institute. Thanks for taking the time to show me around. I wish other Institutes in India will follow your example."

Dr. Chitra Krishnamurti
Scientific Review Administrator
NIH/NHLBI/Divn of Extramural Affairs – RB, Bethesda, MD

"Excellent management, morale and team work at TRC. It is very impressive to see such efficiency and staff involvement across a wide range of professional units."

Dr. Rubina Imtiaz,
Chief, Public Health Systems Development Branch, Div. of Intl. Health, EPD, CDC, Atlanta, USA.

"It was a thrilling and thought provoking interaction with senior scientists and administrators. TRC is doing a commendable job for the country."

Dr. Hardyal Singh
Ex. Director, Research & Medical Education, Punjab.
Consultant, ICMR,
New Delhi

CLINICAL TRIALS

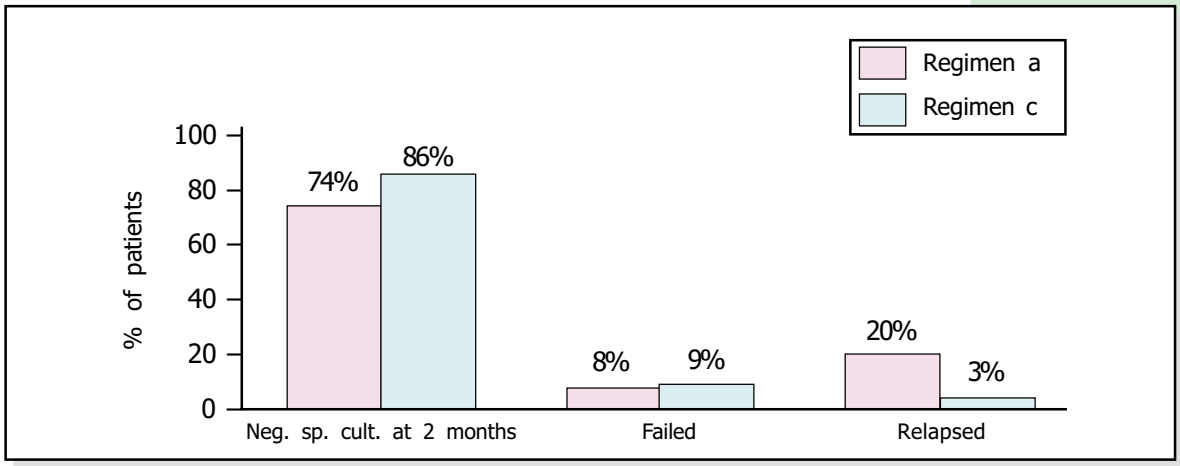
"Sputum culture conversion at two months was lower and relapse rates were higher among patients treated with the intermittent ofloxacin-containing regimen compared to those treated with the control regimen. These interim results suggest that intermittent ofloxacin regimens of 4 months are unlikely to be successful."

1. Ofloxacin-containing ultra-short course regimens for treatment of smear-positive pulmonary tuberculosis patients

In January 2002 TRC published the findings of a randomized clinical trial which showed that a four-month regimen of ofloxacin, isoniazid, rifampicin and pyrazinamide daily for three months followed by isoniazid and rifampicin for one month was successful in 99% of drug-susceptible patients at the end of treatment and relapse occurred in only 4% of patients followed up for 24 months (Indian Journal of Tuberculosis 2002). This was the first clinical trial, which presented compelling evidence that it is feasible to shorten the duration of treatment for smear positive pulmonary tuberculosis, a significant breakthrough in the treatment of tuberculosis. The study, which had 529 patients, also investigated three other regimens, of 3-, 4-, and 5-months duration. Details of the study including results of follow-up for 36-60 months were presented in the Annual Reports of 1999-2000 and 2001-2002

While the results of this clinical trial have been gratifying, the regimens investigated included a daily intensive phase of two to three months of four drugs. The Revised National Tuberculosis Control Programme (RNTCP), is geared for thrice-weekly administration of directly observed treatment. Therefore, if the full benefit of a shorter duration treatment is to be realized then the ofloxacin-containing regimen should be effective when administered three times a week. The regimen can then be dovetailed into the existing RNTCP. With this view, TRC in collaboration with our partners in the state government hospitals and the Chennai Corporation initiated a randomized clinical trial to test the efficacy of intermittent ofloxacin-containing regimens for the treatment of patients with smear positive pulmonary tuberculosis. The study commenced in May 2001 in Chennai and Madurai. The test regimens being studied are ofloxacin, isoniazid and rifampicin three times a week for four months with either pyrazinamide or ethambutol for the first two months; i.e. a) 2 OHRZ thrice weekly/ 2OHR thrice weekly, and b) 2OHRE thrice weekly / 2OHR thrice weekly. These regimens are compared with c) a control regimen of isoniazid and rifampicin three times a week for six months with ethambutol and pyrazinamide for the first two months (2EHRZ thrice weekly/ 4HR thrice weekly). The interim results in 265 patients comprising of drug susceptible and drug resistant patients (167 allocated to regimen a and 98 to regimen c) are summarised in Figure 1. Due to the study design that employed a staggered allocation to the three regimens only 25 patients have been allocated to regimen b so far.

Fig 1: Interim results of a randomised trial comparing intermittent ofloxacin-containing regimens (n=167) with control regimen (n=98) in the treament of patients with smear-positive pulmonary tuberculosis



Regimen a: ofloxacin, isoniazid and rifampicin three times a week for four months with pyrazinamide for the first two months

Regimen c: isoniazid and rifampicin three times a week for six months with ethambutol and pyrazinamide for the first two months

2. Randomised clinical trial to test the efficacy of RNTCP regimens for the treatment of tuberculosis in patients with HIV infection

The aims of this study are: a) to assess the efficacy of RNTCP treatment regimens among HIV-infected persons with pulmonary or lymphnode tuberculosis and to study any additional benefit of an extended continuation phase, b) to study the relationship between stage of HIV disease and response to anti-TB treatment and c) to study the nature of recurrent tuberculosis by using RFLP. The study is being conducted simultaneously in Chennai and Madurai. Patients with pulmonary or lymphnode tuberculosis are randomly allocated to either a 6-month Category I regimen, or to a 9-month regimen. The study commenced in January 2001.

A total of 193 patients have been enrolled to the study. The mean age of the patients is 34 years and 67% had CD4 counts of less than 200 cells/ mm^3 (Figure 2). Among those with positive sputum cultures, 84% were susceptible to all anti-TB drugs, total isoniazid resistance was seen in 13%, while 4% had MDRTB (Figure 3). At 2 months of treatment, sputum smear conversion to negative was observed in 72% of patients and culture conversion in 92%, indicating a satisfactory initial response to chemotherapy. Apart from 10 deaths during therapy among patients treated with Category I regimen, 94% had a favourable response at the end of treatment. Enrollment to the study is continuing.

"Two-thirds of the patients admitted to the trial had a CD4 count of less than 200 cells/mm^3. The drug susceptibility profile of those with positive cultures was similar to that in immunocompetent patients with tuberculosis. Preliminary results indicate that the response to RNTCP CAT I treatment in terms of culture conversion at 2 months and cure rate at the end of treatment are good, in patients with HIV-TB."

Fig. 2: CD4 counts in 170 HIV-TB patients

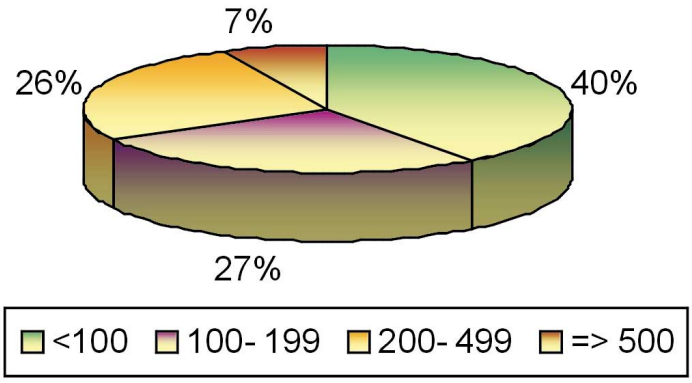
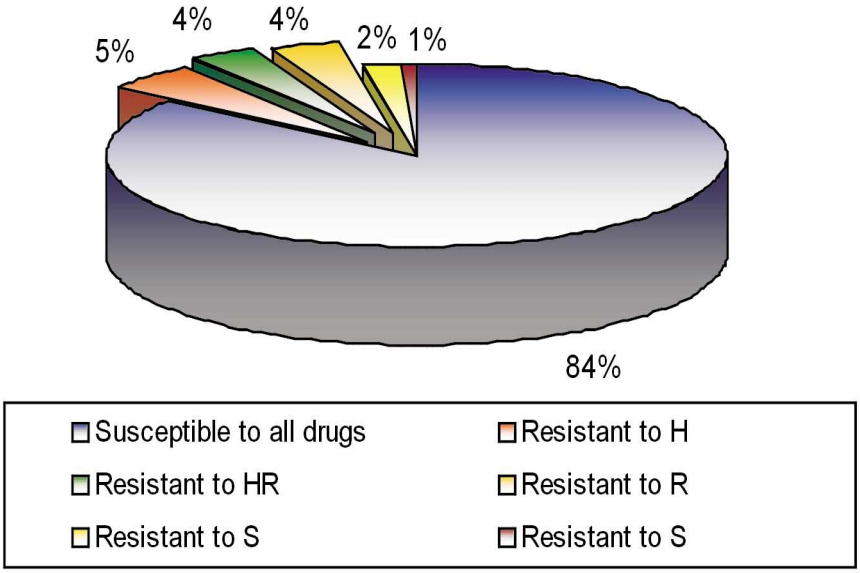


Fig. 3: Drug susceptibility profile of 109 HIV-TB patients



3. Randomised clinical trial to study the efficacy of two regimens in the prevention of clinical tuberculosis in HIV infected persons

HIV infected persons without evidence of clinical tuberculosis are being randomly allocated to receive either isoniazid for three years, or isoniazid and ethambutol for six months. They are then being followed up with clinical, bacteriological and radiological reviews for the development of clinical tuberculosis.

A total of 317 subjects have been enrolled to the study so far. Of these, 103 (68%) are females and the mean age is 29 years (range 18-48 years). Half the subjects had Mantoux test reaction of more than 5mm. There have been three cases of breakdown with culture positive pulmonary tuberculosis while 5 others have had anti-TB therapy instituted on clinical grounds. Enrollment to the study is continuing.

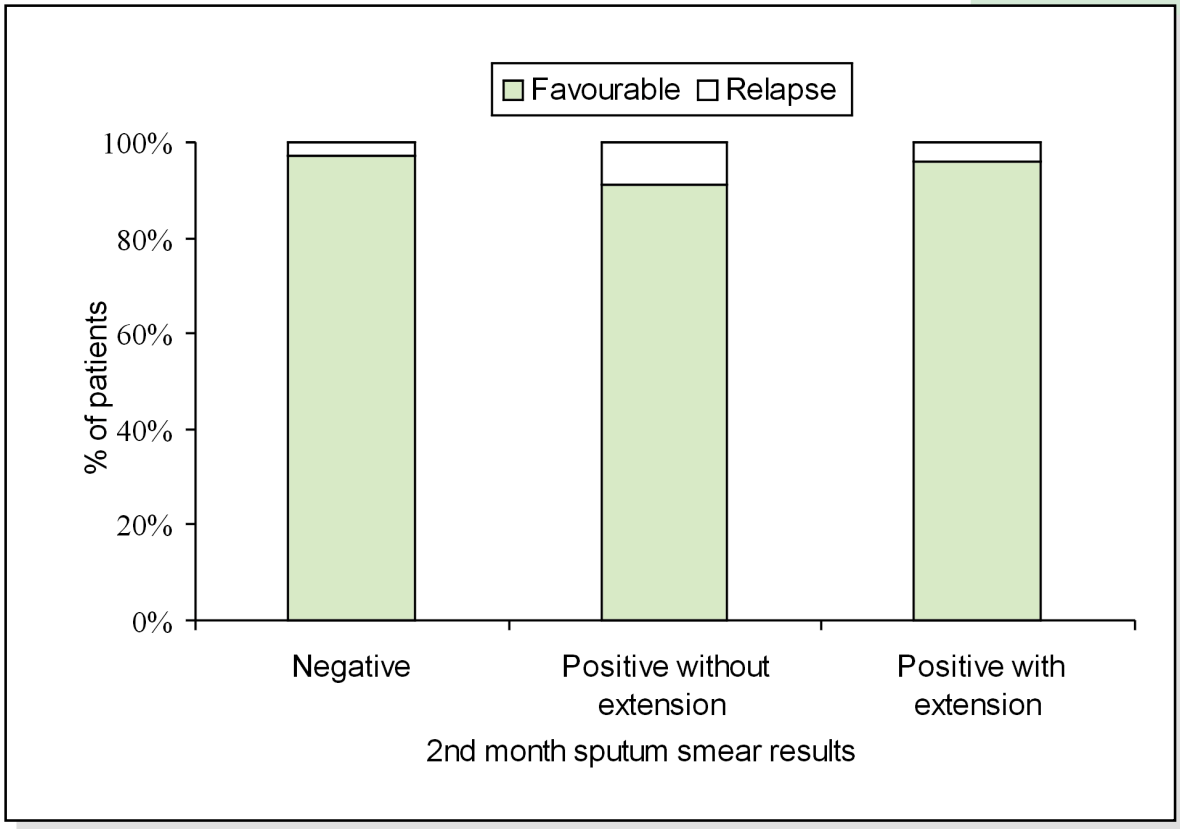
4. Evaluation of an oral 8-month regimen with a non-rifampicin continuation phase for the treatment of sputum positive pulmonary tuberculosis

The primary objective of this study was to evaluate an oral short course regimen with a non-rifampicin continuation phase in the treatment of patients with newly diagnosed sputum-positive pulmonary tuberculosis. The secondary objectives were to study the effect of extension of the initial intensive phase in the presence of 2nd month positive sputum smear, on response to treatment and relapse, and to study emergence of rifampicin and multidrug resistance in patients treated with this regimen.

The follow-up of patients enrolled in this study, (patients were treated with an 8-month regimen of isoniazid, rifampicin, ethambutol and pyrazinamide three times a week for 2 months followed by isoniazid and ethambutol daily for 6 months) is continuing. Of 467 patients enrolled, the relapse rates in those who completed treatment successfully were 3%, 9% and 4% in the groups with second month sputum smears negative, positive without extension of intensive phase and positive with extension of the intensive phase, respectively (Figure 4). The differences were not statistically significant.

"Extension of intensive phase for positive sputum smears at the second month does not significantly influence treatment outcome or relapse. There was no emergence of multidrug resistance in those who failed to respond to treatment or relapsed after successful treatment."

Fig. 4: Relapse rates in patients treated with the 8-month regimen



"Preliminary results indicate that the RNTCP Category 1 regimen of 2 EHRZ thrice weekly / 4 HR thrice weekly is successful in treating patients with newly diagnosed smear positive pulmonary tuberculosis associated with non-insulin dependent diabetes mellitus."

5. A study of Category I regimen of RNTCP for the treatment of patients with sputum-positive pulmonary tuberculosis associated with non-insulin dependent diabetes mellitus

The main objective of this trial was to study the efficacy of Category I regimen of the RNTCP for the treatment of patients with sputum-positive pulmonary tuberculosis associated with non-insulin dependent diabetes mellitus.

Newly diagnosed patients with smear-positive pulmonary tuberculosis and non-insulin dependent diabetes mellitus were enrolled to the study and treated with isoniazid, rifampicin, ethambutol and pyrazinamide three times a week for two months followed by isoniazid and rifampicin three times a week for four months. A total of 100 patients have been enrolled to the study and 75 have completed treatment. Of these 75, 2 (3%) have failed and 3 (4%) have had their treatment changed. Of 52 patients who have been followed up for 12 months, none have relapsed. Interim results thus suggest that the RNTCP Category I regimen may be effective in treating patients with smear-positive pulmonary tuberculosis associated with non-insulin dependent diabetes mellitus.

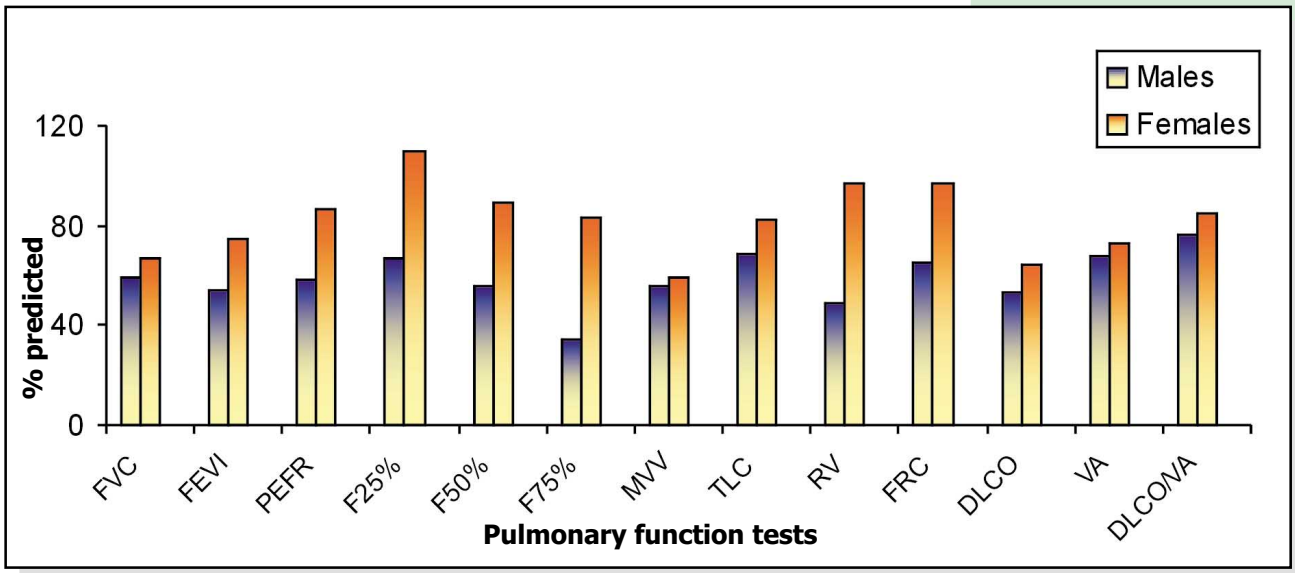
6. Lung function impairment in patients treated for pulmonary tuberculosis and the role of inhaled steroids - a double blind randomized trial

"Of 158 patients recruited to the study, as many as half were found to have abnormal pulmonary function at 3 months of treatment. The reduction in pulmonary function was greater in males compared to females."

An earlier study conducted at our centre revealed that about 50% of patients who had been treated for pulmonary tuberculosis with standard short course regimens had pulmonary function abnormalities (either restrictive or obstructive type of defects). Though the patients were bacteriologically cured of their disease, they continued to have respiratory symptoms suggestive of chronic airway obstruction. Inhaled steroids are anti-inflammatory agents that act topically with minor systemic side effects and are widely used in the management of chronic asthma. The objectives of this study are a) to characterise the physiological and functional changes/ abnormalities in lung function in patients treated for pulmonary tuberculosis and b) to study the effect of inhaled steroids in reducing or reversing the residual defect.

Of 158 patients recruited to the study, 50% were found to have abnormal pulmonary function at 3 months (Figure 5) and have been randomly allocated to receive either inhaled steroids or placebo. Age and height matched controls are being simultaneously studied. The estimated sample size for this study is 200.

Fig. 5: Profile of pulmonary function in TB patients with reduced pulmonary function at 3rd month of treatment (n=31)
(% predicted)



EPIDEMIOLOGICAL STUDIES

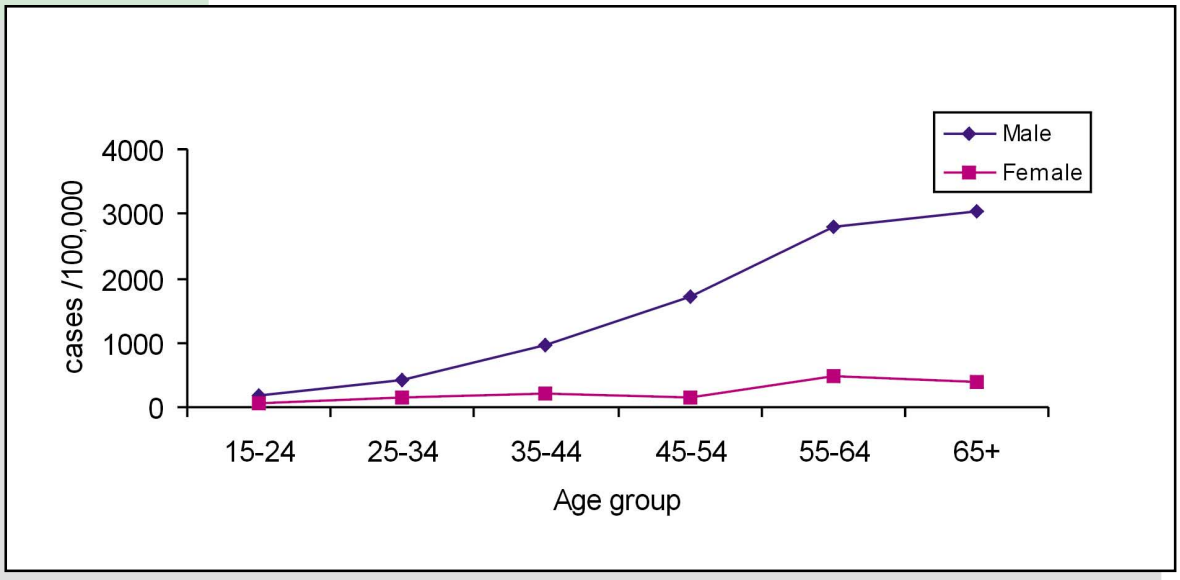
"The prevalence of culture positive pulmonary tuberculosis was 605/ 1,00,000 and was higher in males in all age groups and increased with age."

Community survey of tuberculosis infection and disease

As part of the Model DOTS Project, the TRC is carrying out surveys to estimate the prevalence of tuberculosis infection and disease in Tiruvallur district, covering a population of 5,80,000. The aim of these surveys is to study the trends over time for both infection and disease and thereby to measure the impact of intervention with DOTS strategy in this region. The baseline surveys have been completed and the first resurvey is in progress since June 2001. Of 65,983 subjects targeted for the disease survey, 60,490 (92%) were covered for symptom screening and 59,743 (91%) for X-ray screening. The coverage for sputum collection was 8,562 (96%) of the 8,949 that were eligible. For the tuberculin survey, of 4326 subjects targeted, the coverage was 97%.

Data from the baseline survey (1999-2001) indicates that the prevalence of tuberculosis disease is 605 per 1,00,000 population and increased with age and was higher in males (Figure 6). The male: female ratio was 5.5: 1 for culture positive tuberculosis. The annual risk of TB infection in children aged less than 10 years was 1.8%.

Fig. 6: Prevalence of culture positive pulmonary tuberculosis in Model DOTS area



1. Relapse among Category I patients who have successfully completed treatment in the Model DOTS region

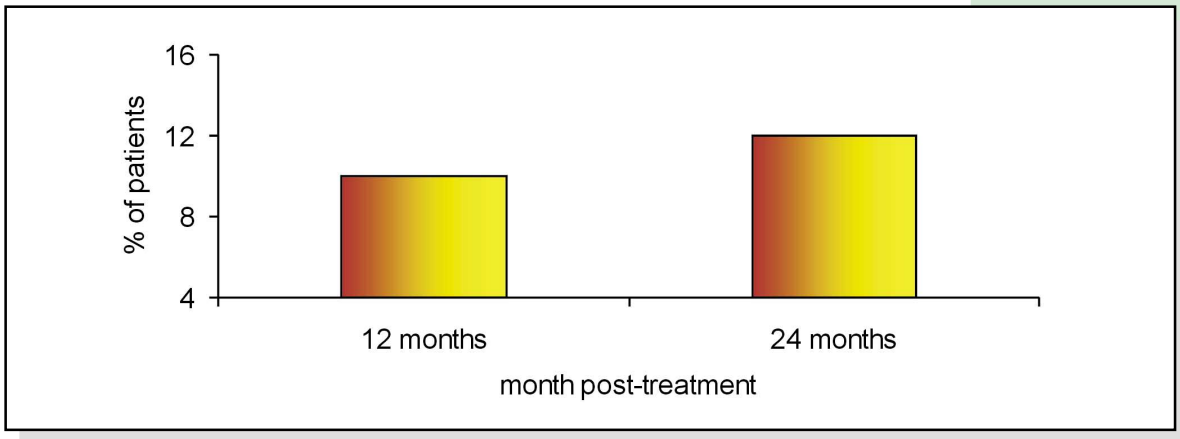
Sputum samples are being collected from patients who have successfully completed treatment from the Model DOTS project area to assess relapse under programme. Sputum specimens are collected at 12, 18 and 24 months and transported to the centre. Specimens are examined by smear and culture for *M. tuberculosis* and drug susceptibility tests are carried out on positive cultures to study the emergence of drug resistance. Relapse is defined as 2 smears positive and / or 2 cultures positive or one smear and one culture positive.

Among 565 patients who were declared cured (2nd quarter 2000 to 4th quarter 2001), sputum was collected from 509 (90%) patients at 12 months and 50 patients (9.7%) had a bacteriologic relapse. At 24 months the relapse was 12% among 193 patients (Figure 7).

Among the 45 patients who had failed treatment, 2 patients had emergence of MDR TB and one of 47 patients who had relapsed had MDR TB at the time of relapse.

"Among patients treated under the RNTCP, relapse rates after successful treatment were 10% at 12 months and 12% at 24 months. Emergence of drug resistance is not a major concern with short-course regimens provided directly observed treatment is practised."

Fig. 7: Relapse rates among successfully treated pulmonary tuberculosis patients at 12 months (n=509) and 24 months (n=193) in Model DOTS region



"Treatment default was positively associated with male sex, age above 45 years and alcoholism. Mortality was independently associated with body weight of < 35 kg and history of previous treatment for tuberculosis."

"The prevalence of multidrug resistance in new patients with pulmonary tuberculosis was <2% and for patients who had previous anti-TB treatment for more than one month was 11%."

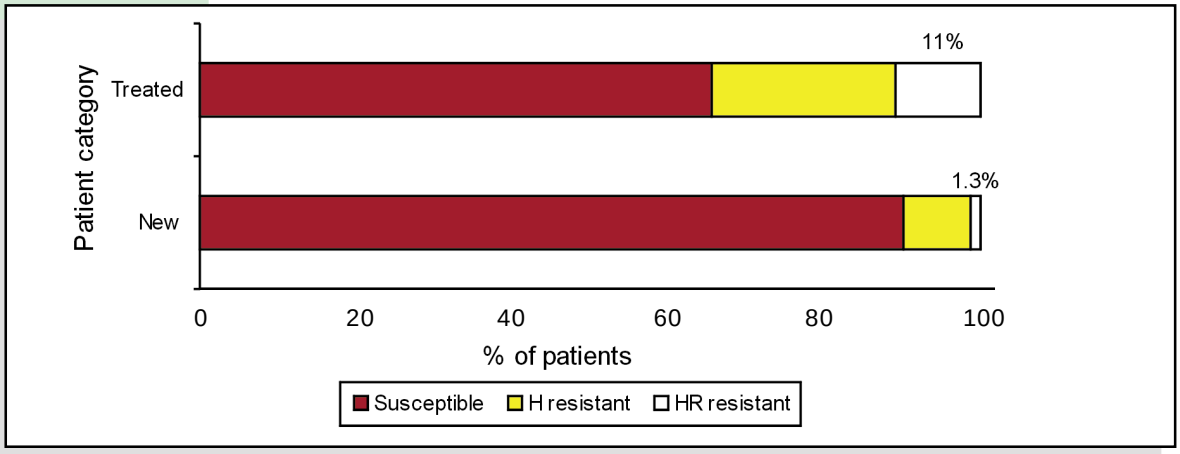
2. Study of risk factors for default, failure and death in RNTCP in the Model DOTS area

A detailed study of the risk factors associated with default, failure, and death among tuberculosis patients treated in the DOTS programme in Tiruvallur district in South India showed that higher default rates were associated with irregular treatment, being male, history of previous treatment for tuberculosis, alcoholism, diagnosis by community survey and age $\geq$ 45 years. Multi-drug resistant tuberculosis patients were more likely to fail treatment. Higher death rates were independently associated with body weight < 35 kg and history of previous treatment for tuberculosis.

3. Prevalence of Multidrug resistant tuberculosis (MDRTB) in the community

The DOTS demonstration and training centre in Tiruvallur has 17 peripheral health institutions where tuberculosis patients are diagnosed and started on treatment. Two sputum samples were collected from patients started on treatment in these centres and tested for culture and drug susceptibility to anti-TB drugs. The results in 1376 newly diagnosed patients and in 327 patients with history of previous anti-TB treatment are illustrated in the Figure 8. MDRTB is less than 2% in newly diagnosed patients.

Fig. 8: Anti-TB drug susceptibility pattern in Model DOTS area



4. A multi-centric study to estimate the prevalence of chest symptomatics attending various health facilities

A multi-centric study was undertaken in 2 districts of Tamil Nadu, one district of Maharashtra and 3 districts of Rajasthan to estimate the prevalence of chest symptomatics among adult outpatients attending health facilities and to compare the efficiency of tuberculosis case detection among patients with cough of different duration (2 weeks vs 3 weeks). The study was conducted in 2 quarters, 4th and 2nd quarter so as to assess seasonal variation if any. The main findings were 55% of the total outpatients attending are new. Prevalence of cough for 2 weeks or more was 4% of the new outpatient department attendance and for 3 weeks or more it was 2.5%. Prevalence of smear positive tuberculosis per 1,00,000 outpatients varied between states, 451/1,00,000 (considering cough of $\geq$ 3 weeks as symptomatics) in Rajasthan, 480/1,00,000 in Maharashtra and 167/1,00,000 in Tamil Nadu. There was no difference between the primary and secondary health care facilities in the rate of sputum positivity (Table 1).

"Case detection can be increased substantially by questioning all out-patients for a history of cough of two weeks or more for selecting patients for sputum smear testing. However, this can be done when RNTCP expansion is completed. Even then, the workload on laboratories and staff need to be considered before implementing the 2-week cough criterion."

Table 1: Proportion of outpatients with chest symptoms and sputum positivity rate at primary and secondary health-care facilities in Rajasthan, Tamil Nadu, and Maharashtra

	New adult outpatients	Cough of 2 weeks or more			Cough of 3 weeks or more		
		CS* (%)	Sputum pos. (%)	Sputum pos. / 1,00,000	CS* (%)	Sputum pos. (%)	Sputum pos. / 1,00,000
Total	**55561**	**4.0**	**12.1**	**481**	**2.5**	**13.3**	**328**
Rajasthan	20856	4.6	17.1	782	2.4	18.5	451
Maharashtra	9582	3.3	15.4	501	1.7	27.5	480
Tamil Nadu	25123	3.8	5.9	223	2.8	6.1	167
Season							
Winter							
Rajasthan	10454	5.8	14.7	851	3.2	15.8	507
Maharashtra	4779	3.5	15.6	544	2.2	25.0	544
Tamil Nadu	11344	4.6	4.4	203	3.5	3.8	132
Subtotal	**26577**	**4.9**	**10.7**	**519**	**3.1**	**11.2**	**354**
Summer							
Rajasthan	10402	3.3	21.3	711	1.7	23.7	394
Maharashtra	4803	3.0	15.2	458	1.3	31.7	416
Tamil Nadu	13779	3.1	7.8	239	2.2	9.1	196
Subtotal	**28984**	**3.2**	**14.1**	**445**	**1.8**	**16.5**	**304**
Primary							
Rajasthan	10074	4.5	15.4	695	2.2	15.5	338
Maharashtra	4791	2.9	7.8	230	1.1	18.5	209
Tamil Nadu	10830	4.5	6.0	268	3.4	6.3	212
Subtotal	**25695**	**4.2**	**10.2**	**428**	**2.5**	**10.5**	**261**
Secondary							
Rajasthan	10782	4.6	18.6	863	2.7	20.8	556
Maharashtra	4791	3.6	21.6	772	2.4	31.9	751
Tamil Nadu	14293	3.2	5.9	189	2.3	5.8	133
Subtotal	**29866**	**3.8**	**13.9**	**526**	**2.4**	**15.8**	**385**

* CS - Chest Symptomatics

"Vitamin A levels were lower in TB patients compared to contacts and normal controls, but returned to normal after successful anti-TB treatment."

5. Estimation of Vitamin A levels among tuberculosis patients

Vitamin A deficiency as a contributing cause to mortality among tuberculosis patients is an issue that is being discussed. Vitamin A levels were estimated in patients with pulmonary tuberculosis before and after treatment, their close contacts without tuberculosis and among healthy volunteers. It was observed that the levels of Vitamin A were lower among tuberculosis patients compared to the contacts and normal volunteers. The levels returned to normal after treatment without vitamin A supplementation.

6. Course of action taken by the smear negative chest symptomatics

In the RNTCP, the diagnostic algorithms for smear negative chest symptomatics required prescribing antibiotics for 2-3 weeks followed by chest X-ray if symptoms persist. The patient is then started on treatment if X-ray is suggestive of active tuberculosis. Currently there is no mechanism to track whether the smear negative patients report back to the health system for further evaluation and whether the diagnostic algorithm is followed strictly.

"Only 50% of sputum smear-negative chest symptomatics reported back to the health facility for review; 28% sought care from another health care provider."

A prospective study was carried out on 523 sputum smear negative chest symptomatics to find out the course of action taken by them and to assess whether the diagnostic algorithm was followed. When a patient was reported as negative after 3 smears and attended to collect the results, a fresh specimen was collected for sputum culture and sensitivity. A month later they were visited at home to find out the action during the one-month period using a semi structured interview schedule. The coverage for interview was 81%. Results are given in Table 2.

Among the 208 subjects who did not reattend the PHC, 41% felt better and 28% had sought care from another provider.

Table 2: Action taken for smear negative chest symptomatics

Total symptomatics	523
Symptomatics interviewed	423 (81%)
Antibiotics received	338 (80%)*
Revisited PHI	215 (51%)
X-ray taken	70 (33%)

* For varying duration of 3 to 7 days

7. Understanding gender inequalities in tuberculosis epidemiology and control

Gender differences with respect to prevalence of tuberculosis infection and disease in the community, access to health services, care-seeking behaviour, diagnostic delay, convenience of directly observed treatment (DOT), stigma, and treatment adherence were studied in the Model DOTS project area.

Data was collected from (i) community survey, (ii) self-referred outpatients seeking care at government peripheral health institutions (PHIs), (iii) tuberculosis suspects referred for sputum microscopy at PHIs and (iv) tuberculosis patients notified under DOTS. Results from the community survey were compared with those from patients notified at PHIs.

The study showed that fewer men than women attended PHIs—68 men for every 100 women. A higher proportion of smear-positive female patients were detected at PHIs than in the community survey (22% versus 13%; p=0.007). The male-to-female prevalence rate ratios for smear-positive patients was 6.5 in the community survey and 4.1 for those notified at health facilities. The probability of notification decreased significantly with age among both men and women. A significantly higher proportion of women than men felt inhibited to discuss their illness with family and friends (21% versus 14%) or felt unwelcome to participate in social events (18% versus 12%). Women were more likely than men to need someone to accompany them for DOT (11% versus 6%; p<0.05). Men had twice the risk of treatment default than women (18% versus 8%; p=0.003) (Tables 3 & 4).

"Despite facing greater stigma and inconvenience associated with DOT, women were more likely to access health services, be notified to the DOTS programme and adhere to tuberculosis treatment than men. Men and elderly patients need additional support to access diagnostic and DOT services."

Table 3: Age-specific initial default rate among smear-positive TB patients detected in the community survey and at primary health institutions, South India, 1999-2001.

Age group (years)	Patients diagnosed in community survey		Initial default rate Number (%)		Patients diagnosed at health facilities		Initial default rate Number (%)	
	Male	Female	Male	Female	Male	Female	Male	Female
15-24	7	0	4 (57)	0 (0)	55	39	4 (7)	5 (13)
25-34	19	8	0 (0)	1 (12)	117	48	12 (10)	6 (12)
35-44	34	6	8 (24)	1 (17)	158	36	30 (19)	5 (14)
45-54	48	5	16 (33)	1 (20)	167	26	25 (15)	3 (12)
55-64	53	9	15 (28)	4 (44)	116	17	18 (16)	3 (18)
65+	36	6	15 (42)	3 (50)	50	4	8 (16)	1 (25)
Total	197	34	58 (29)	10 (29)	663	170	97 (15)	23 (14)

Table 4: Stigma and inconvenience associated with treatment among 1,076 tuberculosis patients registered between November 2000 and December 2001 in a DOTS programme, South India.

	Age < 45 years		Age ≥ 45 years		Total	
	Male (n=340) N%	Female (n=195) N%	Male (n=461)	Female (n=80)	Male (n=801)	Female (n=275)
Found DOT inconvenient	53 (16)	31 (16)	58 (13)	12 (15)	111 (14)	43 (16)
Needed someone to accompany for DOT	8 (2) †	15 (8) †	38 (8) †	16 (20) †	46 (6) †	31 (11) †
Daily activities were affected due to DOT	44 (13)	22 (11)	41 (9)	6 (8)	85 (11)	28 (10)
Lost wages due to DOT§	31 (10)	1 (4)	30 (10)	9 (11)	61 (10)	10 (9)
Faced rejection/ stigma due to DOT	7 (2)	8 (4)	8 (2)	3 (4)	15 (2) *	11 (4) *
Felt unwelcome in social and religious functions	33 (10)	24 (12)	67 (15) ‡	25 (31) ‡	100 (12) *	49 (18) *
Felt inhibited to discuss their illness and treatment with family and friends	72 (21)	43 (22)	41 (9) *	14 (18) *	113 (14) *	57 (21) *

* P< 0.05
† P≤ 0.01
‡ P ≤ 0.001
§ Limited to employed patients only
DOT denotes directly observed treatment

8. Involving private practitioners in the RNTCP

A feasibility study to involve private practitioners in the RNTCP has been initiated in May 2001. After listing the private laboratories in Tiruvallur area, 10 Laboratory Technicians from the private laboratories were trained in sputum microscopy using RNTCP modules. There were 78 allopathic medical practitioners in the area and they were given orientation to RNTCP on 2 occasions followed by focus group discussions to formulate the study design. The practitioners were met on one to one basis and were given a referral book of sputum examination forms with serial numbers. They were given the option of referring the suspects either to one of the private laboratories or to the government hospital at Tiruvallur. For patients diagnosed, the treatment cards were made in duplicate and the first dose of treatment was given at the tuberculosis unit (TU). Further management of the patient was decided in consultation with the private practitioner. The treatment box was handed over to the DOTS provider and the provider was trained in administration of treatment and treatment card maintenance. A total of 48 private practitioners have been enrolled. 614 symptomatics have been referred, 146 have been diagnosed to have tuberculosis: 93 smear positive, 42 smear-negative and 11 extra pulmonary, and all have been started on treatment.

SOCIOLOGICAL STUDIES

1. A study of HIV/AIDS awareness in relation to risk behaviour in HIV infected persons

The aim of this study was to find out if awareness of HIV/AIDS influences change in high risk behaviour and to find out the reasons for continued high risk behavior in spite of awareness of HIV/AIDS. Information was obtained in 108 HIV positive individuals using a semi-structured questionnaire and narrative summaries. Eighty four percent of the respondents in this study were in the age group of 25-44 years. Awareness of HIV was very high with more than 80% of respondents saying they were aware of HIV and that it was through multi-partner sex. Gender and literacy were not barriers to awareness. Seventy four percent of the respondents continued risk behaviour in spite of being aware of HIV/AIDS. Women experienced a sense of helplessness, exploitation and harassment. In India simply being married is a risk factor for HIV as the transmission from husbands to wives is frequently observed. Among the men, reasons for continued high-risk behavior in spite of awareness were peer pressure, sexual desire, alcoholism and various misconceptions. This study endorses the fact that knowledge alone does not and will not result in behavioral change. The findings call for a need to address these issues in preventive programmes aimed at controlling the spread of HIV.

2. A study of condom acceptability among men in an urban population in South India

This study examined the acceptability of condoms among men from Chennai, South India. A sample of 150 male respondents who had at least one risky sexual experience were interviewed. The respondents included HIV positive and HIV negative individuals attending the STD clinic, college students and homosexuals. Awareness on condom usage was high and 83% had used condoms at least once. The most common reason for using condoms was protection from disease (43%), protection from HIV (20%) or partner insistence (25%). However, dissatisfaction with condom usage was expressed by 79% of respondents. Two of the prime target groups for condom promotion programmes are truck drivers and salesmen, who travel out of home over long periods and their vulnerability to risky sexual exposure is high. Students are a sexually vulnerable group. Sexual dissatisfaction with condom use was also expressed among half of the HIV positive respondents and this is also a reason for concern. Of the respondents who used condoms for the first time, 72% were HIV negative compared to 34% among those who did not use condoms. This study showed that dissatisfaction with condom use was high in groups studied. This is a cause for concern as dissatisfaction could lead to stoppage or discontinuing condom use. The findings highlight the need for condoms to be more 'user friendly' with good quality condoms made readily available and accessible, if condoms are to be accepted.

"As many as 79% of respondents in this study expressed dissatisfaction with condom use. This is a cause of concern as dissatisfaction may lead to discontinuance. There is a need for condoms to be made more user-friendly with good quality condoms being made readily available and accessible."

LABORATORY STUDIES

"Drug resistance to isoniazid, either alone or in combination with other anti-TB drugs was observed in 17% of patients and to isoniazid and rifampicin (MDRTB) in 1%."

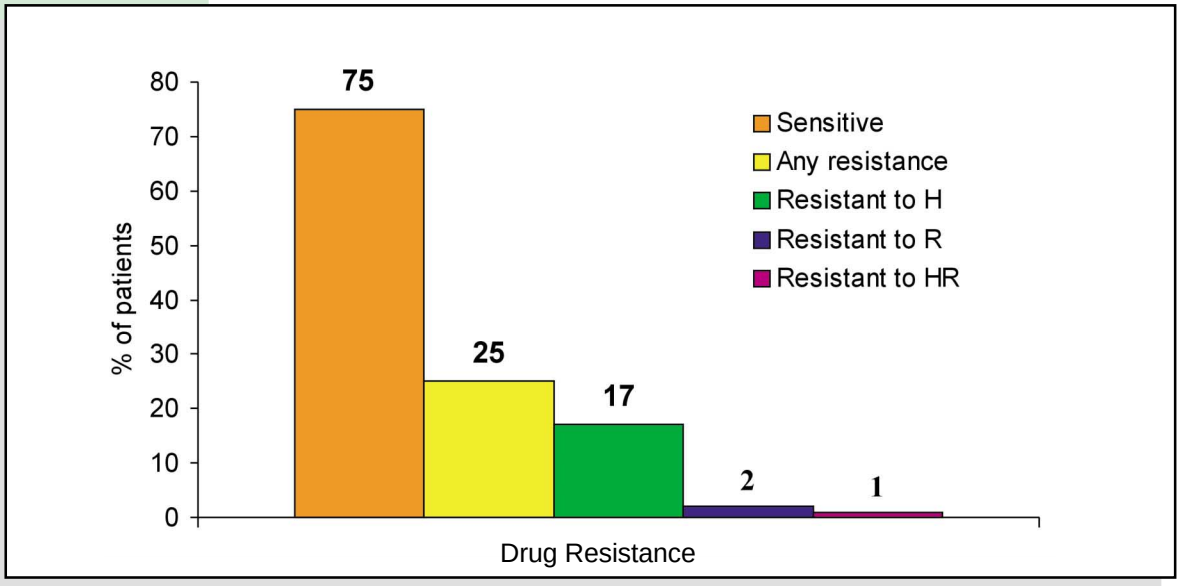
1. Studies on anti-TB Drug Resistance Surveillance in four districts

As per the recommendation made by the Expert Group (organized by the Central TB Division held in September 1997) on Drug Resistance Surveillance (DRS), systematic studies on DRS in newly diagnosed TB patients were undertaken by the centre according to the WHO/IUATLD Global DRS protocol in order to provide baseline data on drug resistance at few sites in India. These studies were conducted in North Arcot (Vellore and Thiruvannamalai districts – Tamil Nadu), Raichur (Koppal and Raichur districts – Karnataka), Wardha (Maharashtra) and Jabalpur (Madhya Pradesh). These districts were chosen since baseline drug resistance information was already available from these districts because of studies conducted already either by the centre or with ICMR/TRC assistance. It was expected that a resurvey would enable an understanding of the trend in resistance pattern over the years.

Detailed outcome of results obtained in North Arcot and Raichur and Wardha districts were given in the previous year Annual reports.

The present report details DRS data in the predominantly tribal population of Jabalpur District. The available data from 273 patients revealed that 75% patients harboured fully drug susceptible organisms and remaining 25% of the patients had shown resistance to one or more anti-TB drugs and MDRTB was observed in 1% (Figure 9).

Fig. 9: Primary drug resistance in Jabalpur district (n=273)



2. Determination of susceptibility to gatifloxacin, a third generation quinolone, against *M. tuberculosis* using four different test methods

The aim of this study was to determine an acceptable *in vitro* definition of resistance to gatifloxacin using different susceptibility testing methods. Ten strains of ofloxacin susceptible and 10 strains of ofloxacin resistant *M. tuberculosis* were tested by the absolute concentration method on LJ and by proportional susceptibility method on LJ, 7H11 as well as by BACTEC radiometric method for different concentrations of the drug.

The MIC of gatifloxacin on LJ medium was 0.5 – 1.0 µg/ml by the absolute concentration method. Using a well accepted criterion of 1% or more growth as a definition of resistance by proportion method, a concentration of 0.5 µg/ml in LJ and 7H11 and a concentration of 0.25 µg/ml in BACTEC radiometric method yielded 100% agreement with the absolute concentration method.

"In vitro definitions of resistance to gatifloxacin have been determined by various methods – MIC : 0.5-1.00 µg/ml, Proportion method (LJ & 7H11): 0.5 µg/ml, BACTEC : 0.25 µg/ml"

3. *In vitro* activity of Quinolones against *M. tuberculosis*

The aim of this project was to study the *in vitro* activity of different quinolones like gatifloxacin, sparfloxacin, lomefloxacin, ciprofloxacin, and ofloxacin by absolute concentration method and to look for any cross resistance. 55 strains of *M. tuberculosis* isolated from same number of patients were tested for susceptibility by absolute concentration method on LJ and 7H11 medium for different quinolones.

1. Fluoroquinolones exhibited cross resistance at different levels.
2. Among the quinolones tested, gatifloxacin showed a low mean MIC on both ofloxacin resistant and sensitive strains.
3. Activity of quinolones on both ofloxacin sensitive and resistant strains were in the order of gatifloxacin > sparfloxacin > ofloxacin ≥ ciprofloxacin >lomefloxacin (Figures 10 & 11).

"Gatifloxacin has shown pronounced in vitro activity compared to other quinolones tested. Though quinolones exhibited cross resistance at different levels, even the strains resistant to ofloxacin have shown a much lower MIC to gatifloxacin."

Fig. 10: Inhibition of susceptible *M. tuberculosis* strains by Quinolones

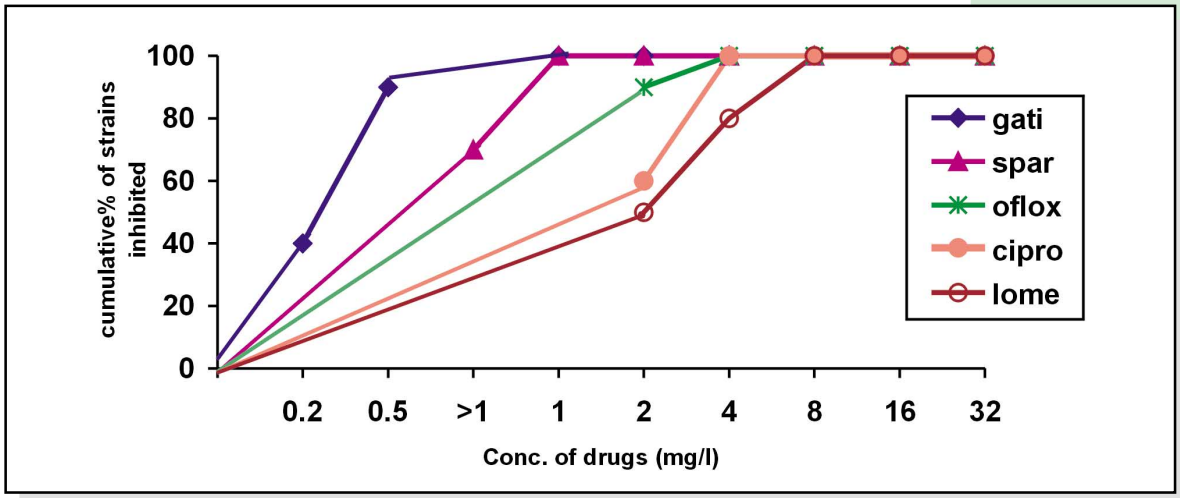
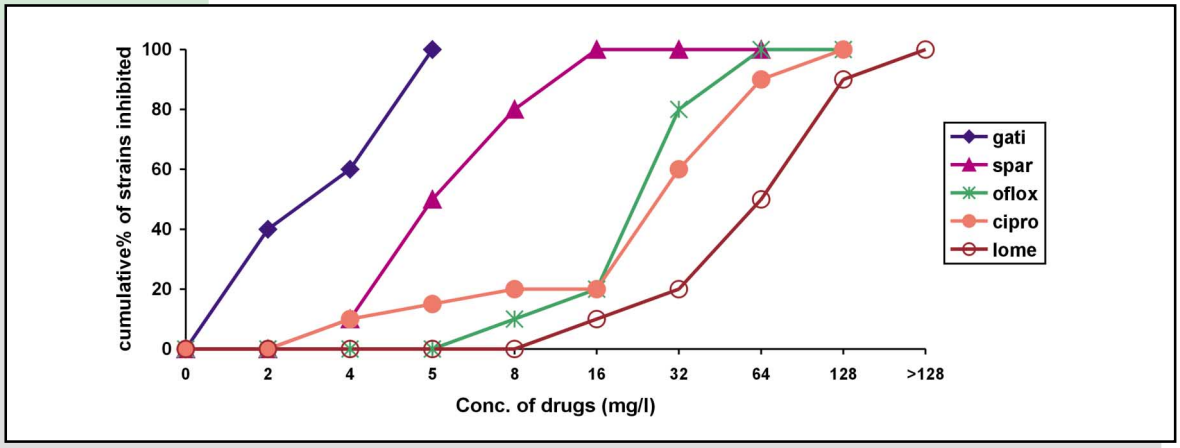


Fig. 11: Inhibition of resistant *M. tuberculosis* strains by Quinolones



4. Rapid pyrazinamide sensitivity testing of *M. tuberculosis* clinical isolates using luciferase reporter phage assay

A rapid sensitivity assay for performing drug sensitivity test for pyrazinamide was standardized in this study. As no standard conventional test is available, pyrazinamidase test was used as the gold standard. Using a thick suspension of primary culture on LJ, luciferase reporter phage (LRP) assay was done on the same day for 35 *M. tuberculosis* clinical isolates after incubating them with phage for 3 to 6 hours with and without drug. Thus, by combining the assay phase with drug treatment phase, we aimed at measuring the action of drug on metabolizing cells. Secondly, the same LRP assay was done on the 3rd day. This approach was to find out whether the drug acts on the multiplying cells or not. It was found that the drug acted both on metabolizing and dividing cells. Figures 12 and 13 show the sensitivity and specificity of 0 day and 3rd day LRP as compared to 4th and 7th day pyrazinamidase tests. The maximum agreement was seen with 7th day pyrazinamide and same day LRP. It is planned to do the assay for a total of 100 clinical isolates.

Fig. 12: Comparison of PZA-LRP against 7th day PZase

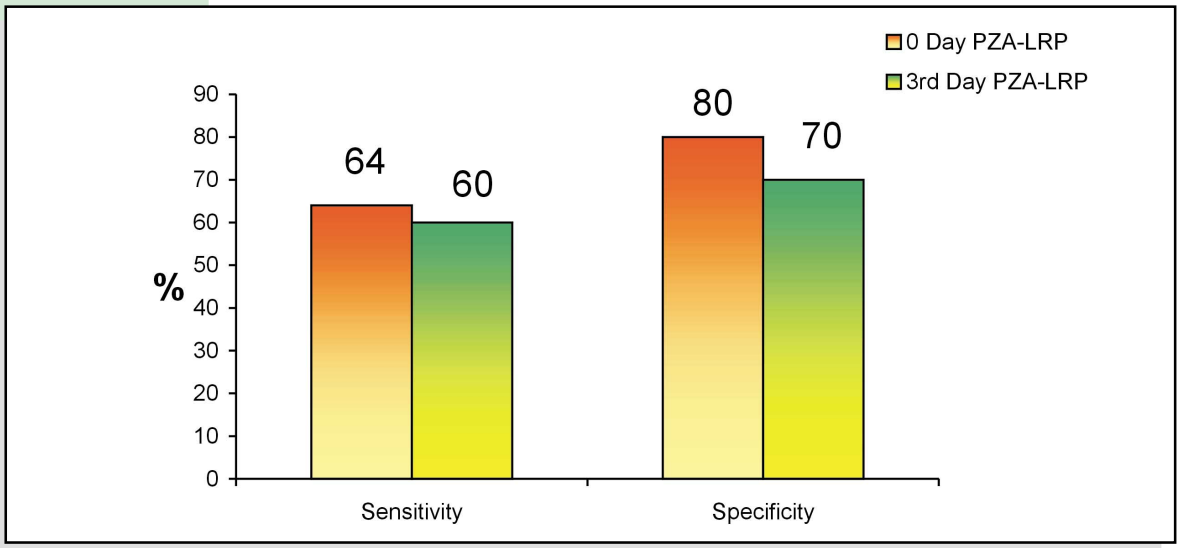
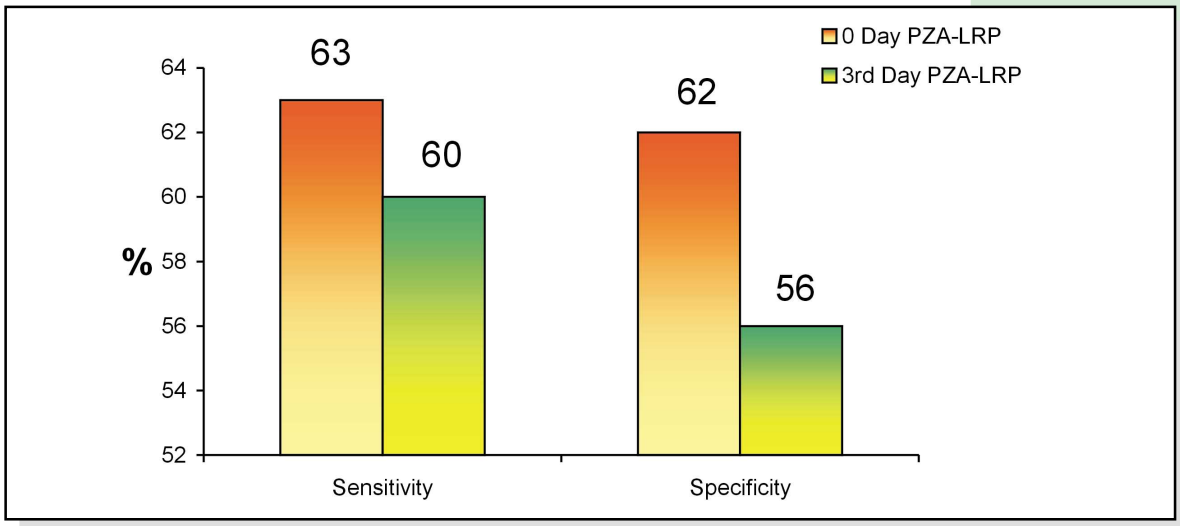


Fig. 13: Comparison of PZA-LRP against 4th day PZase



5. Antibacterial and antimycobacterial activities of compounds from fruits of *Piper longum*

Piper longum has been suggested for use as a tonic in the ancient Ayurvedic system of medicine. Piperine, an alkaloid isolated from *Piper longum* is reported to act as a bioenhancer. Both piperine and *Piper longum* are suggested to have a hepatoprotective effect. Piperine is also reported to act as a bioavailability enhancer. Hence a detailed study was planned. The active compounds were extracted from the *Piper longum* fruits and Compounds Y and P and a white powder were obtained.

UV, IR and NMR spectra of compounds Y and P were recorded and compared with spectrum of commercial piperine. By comparison of spectra, it was concluded that compound P resembles piperine and compound Y has a closely related molecular structure to compound P.

The antibacterial activity of the white powder was assessed. At a high concentration of 1.14 mg/ml, the white powder showed 70-80% inhibition of *E.coli* and *S.aureus*. At a concentration of 571 µg/ml, white powder showed no inhibition of *M. smegmatis* (Table 5).

Table 5: Antibacterial activity of *Piper longum* extract

	E.coli		*S. aureus*	
	Concentration of Y		Concentration of Y	
	115 µg/10 µl	230 µg/10 µl	94.5 µg/10 µl	189 µg/10 µl
Zone of Inhibition (cms)	3.89	5.03	2.80	3.81

Compound Y gave a minimum zone of inhibition at a concentration of 100 µg against *E.coli* and *S.aureus*. These findings indicate that compound Y has antibacterial activity but does not have antimycobacterial action. Compound P has antibacterial and antimycobacterial activity at high concentration.

Studies have been initiated in animal models to study the following aspects.

a. Hepatoprotective effect of *Piper longum* and Piperine

b. Effect of co-administration of *Piper longum* with anti-TB drugs in infected experimental animals.

6. Reduced detection by ZN method of AFB in sputum samples preserved in cetylpyridinium chloride

A study was carried out to estimate the sensitivity of ZN method of staining for detection of AFB in sputum samples preserved in cetylpyridinium chloride (CPC) solution. Duplicate smears, prepared from each of the 988 sputum samples collected in CPC solution were randomly allocated to different methods and read blind. All the samples were processed for culture of *M. tuberculosis*. Comparison of the results of the ZN method and the auramine-phenol method is shown in Figure 14. Comparison of the results of ZN smears and auramine-phenol stained smears with the culture results is shown in Figure 15.

"Sensitivity of ZN method in detecting AFB is significantly reduced in sputum samples preserved in CPC solution."

Fig. 14: Comparision of ZN and FM methods to detect AFB

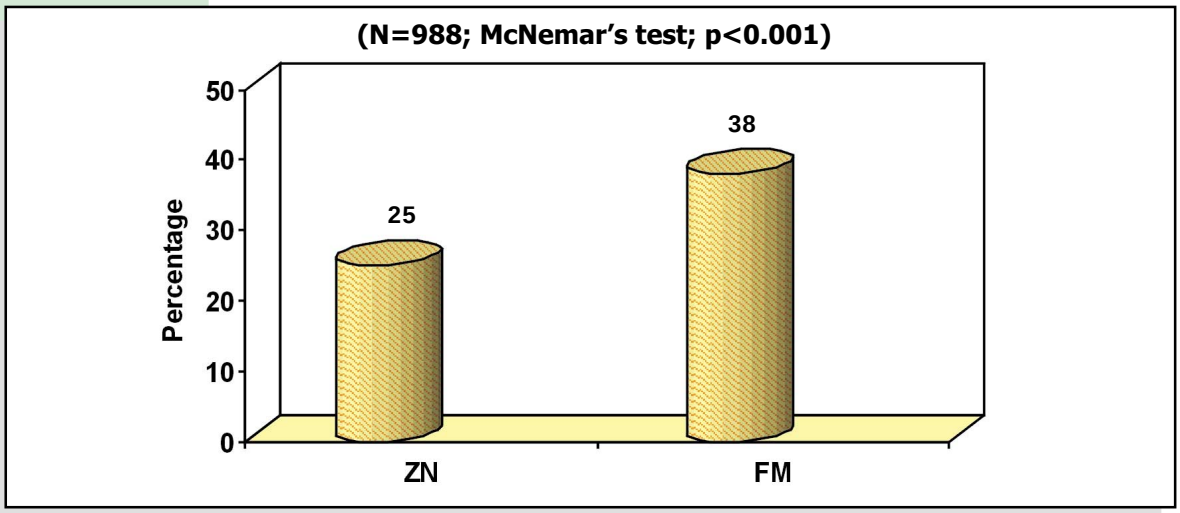
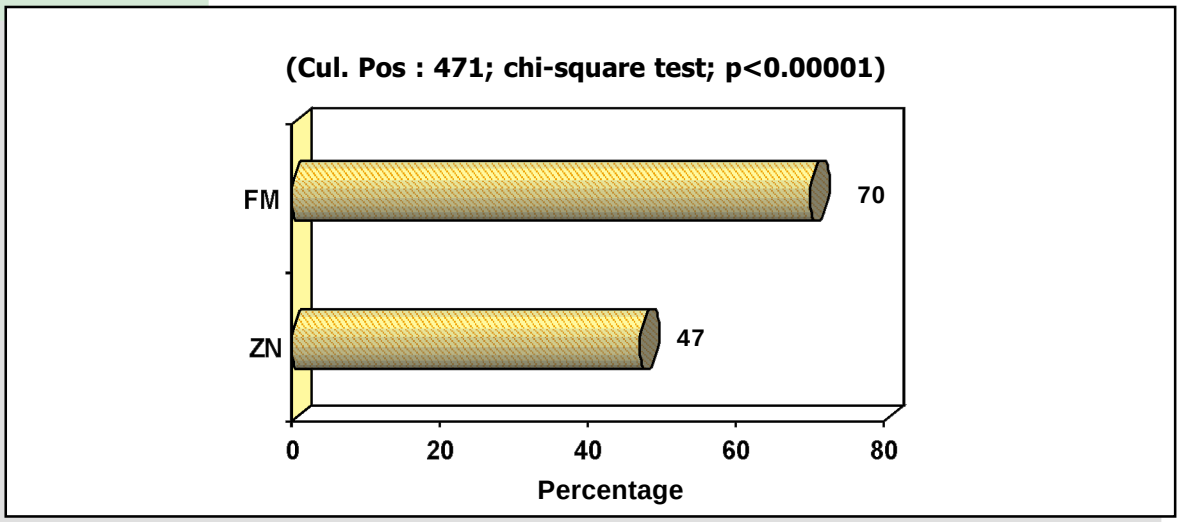


Fig. 15: Comparision of ZN and FM methods in culture positive specimens

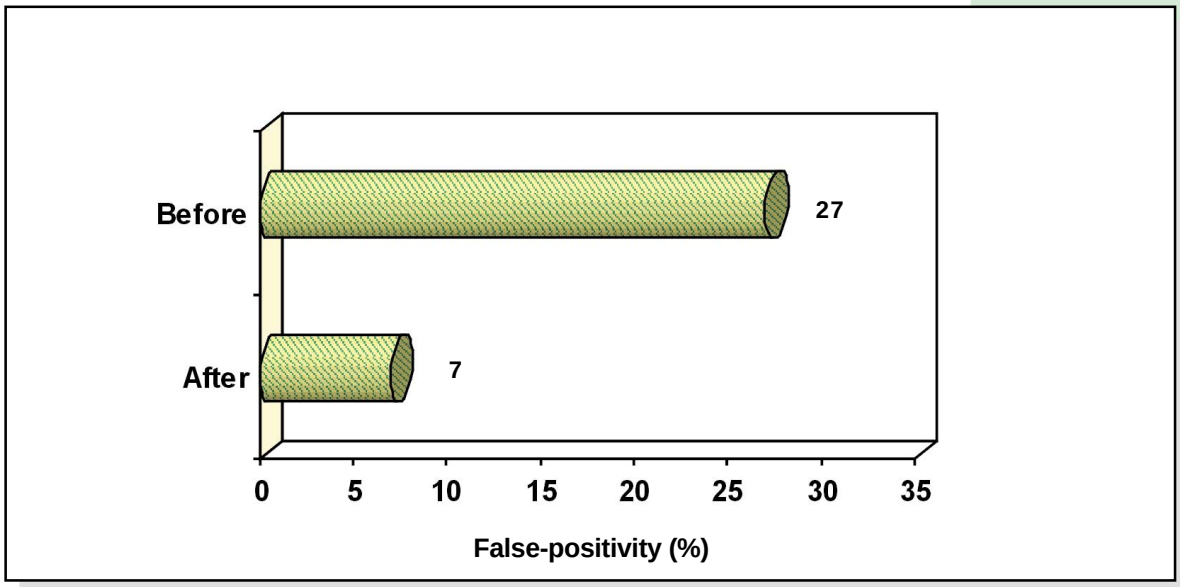


7. Re-staining of smears to assess the false-positive errors at the peripheral centres in quality assurance of sputum AFB microscopy

As prolonged storage of smears under sub-optimal conditions was found to result in increased false-positive errors, 422 sputum smear slides (103 positive and 319 negative), that were not stored properly in the fourth quarter of 1999, were read in the first quarter of 2000 with and without re-staining. The results are shown in Figure 16.

"Re-staining of slides before re-reading by the controller is essential to assess the performance of LTs at the peripheral centres."

Fig. 16: False-positives before and after re-staining of slides (Pos. 103; Neg : 319; McNemar's test; p<0.001)



8. Comparison of 0.3% and 0.1% basic fuchsin with standard 1% carbol fuchsin in ZN staining

In the standard Ziehl-Neelsen method of staining, basic fuchsin is being used at a concentration of 1%. A study is being carried out to determine the optimal concentration of basic fuchsin to be used for staining. Basic fuchsin at concentrations of 0.3% and 0.1% are being used and compared with the standard method. In a sample of duplicate smears from 356 samples, no significant difference was observed between 0.3% and 1% basic fuchsin indicating that 0.3% basic fuchsin is as sensitive and specific as 1% basic fuchsin in ZN staining. The comparison of smear results of ZN method using 0.1% and 1% basic fuchsin indicates that there was a significant loss of smear positivity in 0.1% method.

"Use of 0.1% basic fuchsin will significantly reduce the rate of smear positivity."

9. A study on the safety of unstained AFB smears

Prefixed and unstained sputum smears are used routinely under field conditions in panel testing. Unless the smears are made safe to carry, this procedure might pose a potential hazard. Therefore, a study is in progress to examine the presence of viable tubercle bacilli in smears that are fixed following a routine RNTCP procedure by flaming or by placing over a hot plate at 80^0C for 10 minutes as practised at this centre. These procedures are compared with sputum smears that are fixed in a reagent containing 5% phenol in 70% ethanol. The viability of the bacilli present in these three different preparations is being examined by culturing the materials of the smear content in vancomycin containing media such as LJ and selective Kirchner's liquid medium and also in BACTEC 12B vials. This study is in progress.

10. Evaluation of BIO FM Kit for diagnosis of *M. tuberculosis*

BIO FM test kit based on Middlebrook 7H9 broth with an indicator, a method recently developed by BIO-RAD was evaluated to determine its sensitivity in detecting the positive growth of *M. tuberculosis* and also the relative turn around time required for the same. As controls, aliquots made from the same sputum samples were also cultured on LJ, 7H11, and BACTEC 460 system. A total of 48 sputum samples were included in this study. The total number of positives isolated by any one method was 24. The number of positives isolated by BIO FM, LJ, 7H11 and BACTEC were 15, 17, 20 and 21 respectively and the average number of days taken for reporting positives was 15, 21, 16 and 10 respectively (Table 6). The paired sample t test with respect to the days of positivity obtained showed a significant difference between BACTEC vs BIO FM methods (n-14, P-<0.01). BACTEC method as expected showed better results with less turn around time compared to BIO FM. However, the difference between BIO FM and 7H11 method was not significant (n-12, P=0.1) and the BIO FM method showed significantly better results compared to LJ (n-13, P=0.001).

To conclude, although less number of positives were obtained in BIO FM kits compared to other methods, the difference in the number of positives obtained between the methods and the total number of samples processed were minimal. The average number of days taken to report as positive was in the order of BACTEC < BIO FM < 7H11 < LJ.

"BIO FM may be an alternative to the conventional LJ method in places where BACTEC and other recent methods cannot be afforded."

Table 6: Number of positives and number of days for reporting positives by different methods

Methods	Total no. of positives	No. of days	
		Range	Mean
BIO FM	15	2 – 22	15
LJ	17	11 – 42	21
7H11	20	7 – 25	16
BACTEC	21	2 – 32	10

11. Nitrogen laser irradiation of *M. tuberculosis*

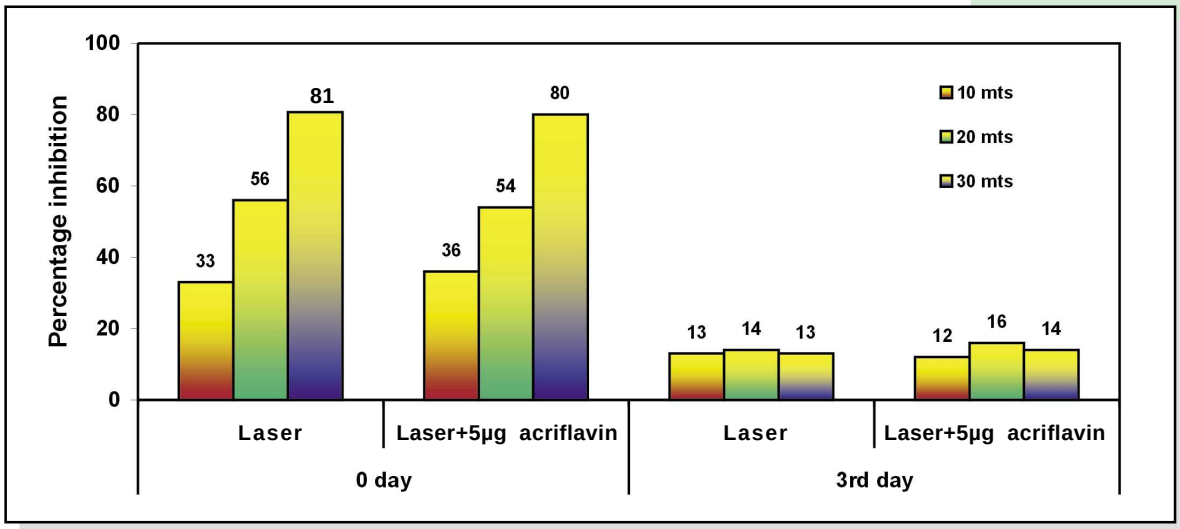
Nitrogen laser has dose dependant killing effect on *M. tuberculosis*. The clinical isolates of *M. tuberculosis* were subjected to the action of nitrogen laser. The viable organisms in samples taken at 0, 10, 20 and 30 minutes of laser exposure were measured by luciferase reporter phage assay and expressed as Relative Light Units (RLU). There was a dose dependant reduction in viability with a maximum of 80% killing as compared to the control at 30 minutes. Samples further incubated and assayed on the third day showed an increase in viability reaching the level of the control.

We further investigated whether the damage is done at DNA level or not. Two experiments were done to explore this fact. Acriflavin, the DNA inhibitor, was used in the first experiment. Using 5 µg acriflavin and 0.5 OD culture suspension, the results obtained with 7 clinical isolates indicate that the damage is not at the DNA level. In other words, the vials with and without acriflavin had equal number of viable cells (Figure 17).

Viscometric assay of DNA cleavage was done to confirm the above finding. Damage at the DNA level increases the viscosity of the culture. Neither cultures tested showed increase in viscosity, thus reconfirming the fact that nitrogen laser does not lead to DNA damage in *M. tuberculosis.*

Increase in viability reaching almost the level of the control on the third day suggests that there is a repair mechanism playing a role when allowed to grow in 7H9 medium after laser exposure.

Fig. 17: Effect of laser alone and with 5 µg Acriflavin on seven *M. tuberculosis* clinical isolates at 0.5 OD as assayed on 0 day and 3rd day by LRP assay



12. Development of Luciferase Reporter Phages from new Chennai phages

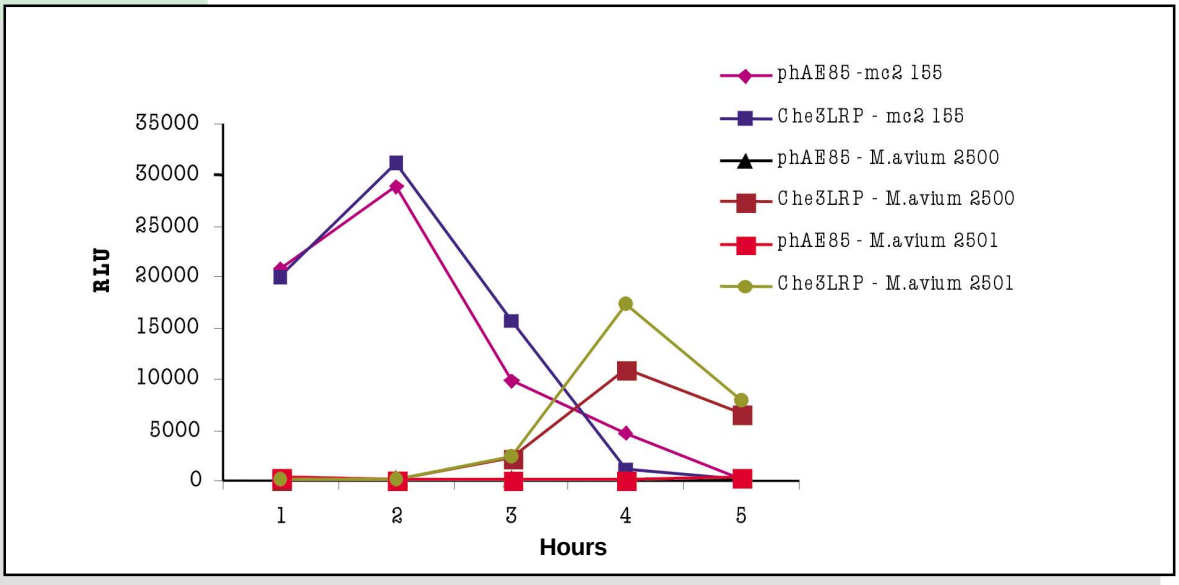
Luciferase Reporter Phage (LRP) assay is a simple, rapid and highly specific assay to measure cell viability. However, its sensitivity is very low, requiring 10^4 organisms/ml, even with the third generation LRPs developed from mycobacteriophages TM4 and D29.

Indigenous phages isolated from enriched soil samples in Chennai (Che phages) were characterized. The unique ones were selected to obtain better phage constructs to improve the sensitivity of the assay. Construction of LRPs was attempted at Albert Einstein College of Medicine, New York with lytic phages Che3 (infecting *M. avium*), Che7 (infecting *M. tuberculosis)* and Che11 (infecting *M. tuberculosis* and *M. intracellulare*) along with a temperate phage BXZ2 which infects *M. tuberculosis*.

LRP was generated in two steps. A cosmid vector is inserted randomly in a nonessential region of the phage genome to obtain a shuttle phasmid. Replacing the vector with another containing the fire fly luciferase gene (Fflux), yield the reporter phage. The *E.coli* cosmid vector used in the first step was pYUB328. A random library of the phages was constructed using *Sau*3A I partial digests of the phage DNA. Mycobacteriophages Che3 and Bxz2 yielded the shuttle phasmids whereas no viable phage could be obtained with Che7 and Che11 cosmid library.

The LRPs of Che3 and Bxz2 were obtained by replacing the vector pYUB328 with pYUB556, which contains the Fflux gene driven by hsp60 promoter. The light producing kinetics of these constructs were checked by incubating 10^6 cfu/ml of organisms with 10^7 pfu/ml of the reporter phages. For the Che3-LRP, two strains of *M.avium*, $mc^2$2500 and 2501 and *M.smegmatis* $mc^2$155 were used. For BXZ2-LRP, *M.bovis* BCG and *M.smegmatis* $mc^2$155 were used (Figures 18a & 18b).

Fig. 18a: Light production kinetics of Che3-LRP



Phages Che 7 and Che 11 could not produce shuttle phasmids, possibly because their DNA was large for *in vitro* packaging in lambda phage. The reporter phage of BXZ2 proved to be less sensitive. This is possibly because the phage is not a true lysogen.

Subsequently, a similar attempt to construct LRP with a true lysogenic phage Che12 yielded promising constructs designated as phAETRC11-20. Figure 19 shows the RLU production of these constructs as compared to phAE129 and phAE85 with *M. bovis* BCG. The two constructs phAETRC11 and phAETRC16 are promising. The LRP construct phAETRC2 of Che3 is being checked for light production with *M. avium* clinical isolates. Similarly, experiments are on to test smear positive sputum samples with phAETRC11 and 16 of Che12. The study is ongoing.

Fig. 18b: Light production kinetics of Bxz2LRP

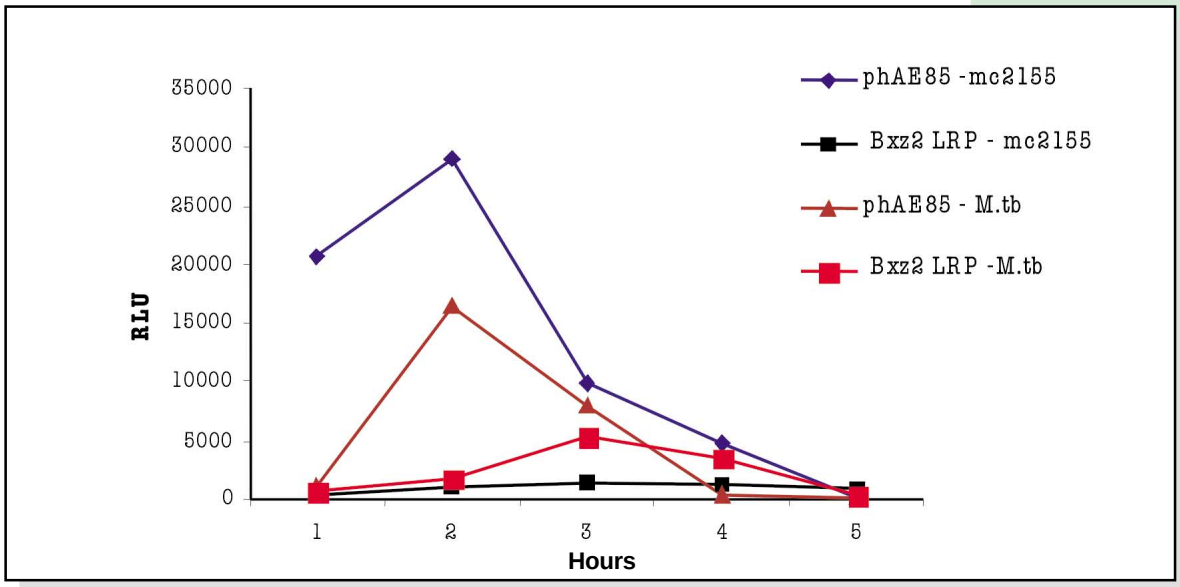
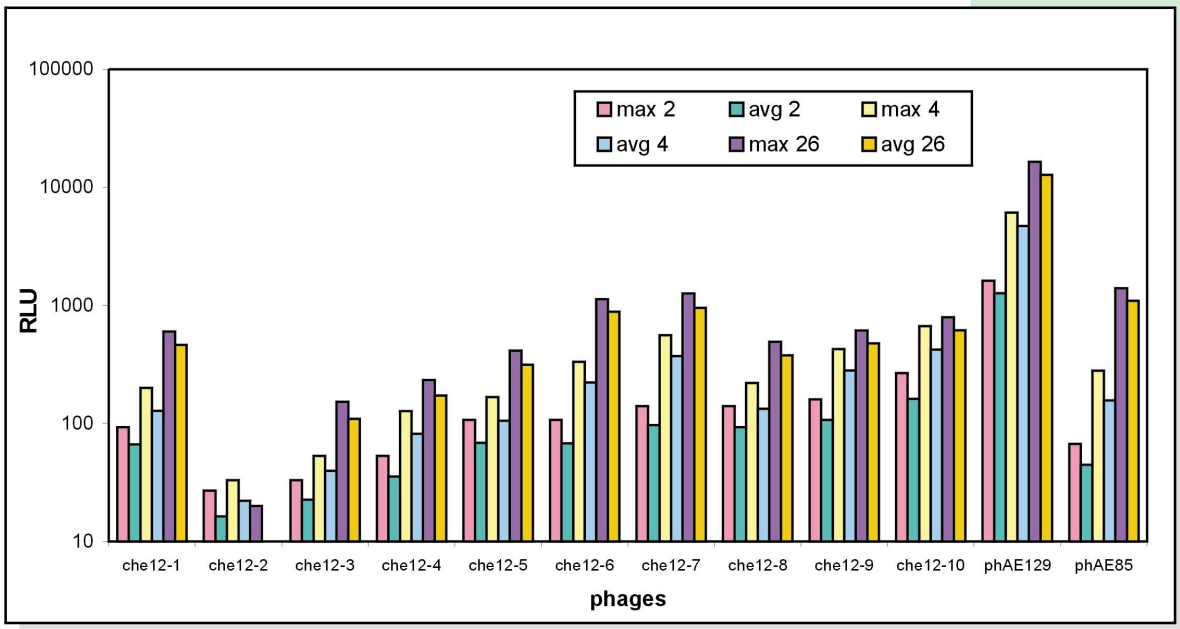


Fig. 19: Light production kinetics using BCG-Pasteur

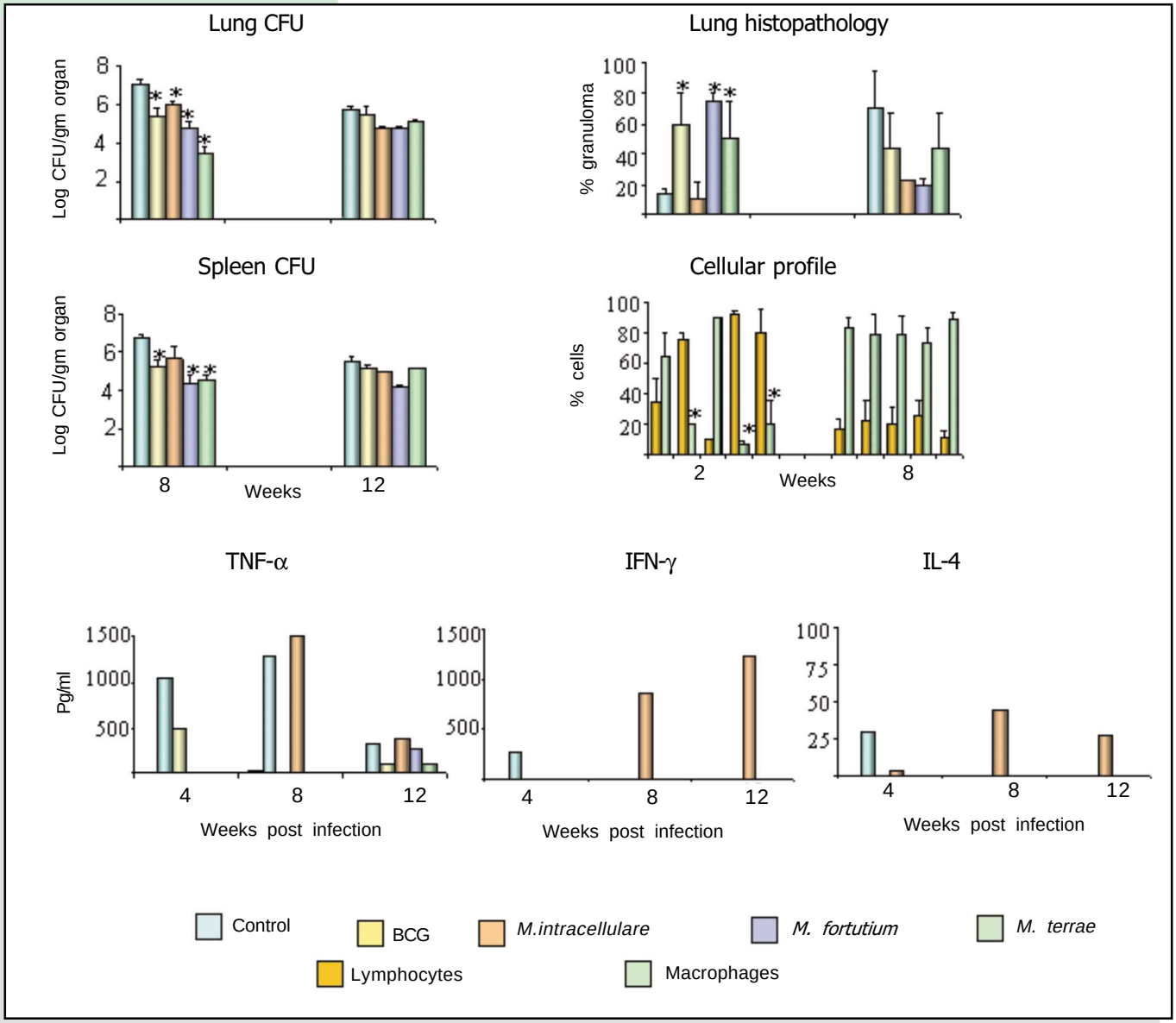


"Based on bacteriological, histopathological results and cytokine levels, it appears that M. intracellulare interferes with the protective efficacy of BCG."

13. Effect of non tuberculous mycobacteria on the protective efficacy of BCG in murine tuberculosis

Previous exposure to non tuberculous mycobacteria (NTM) in the environment is considered as one of the reasons for the failure of BCG vaccine to give protection against pulmonary tuberculosis in south India. To test this hypothesis, mice were sensitized orally with *M. intracellulare*, *M. terrae* and *M. fortutium*, the most common NTM in the environment of south Indian BCG trial area, and then immunised with BCG. The effect of NTM on BCG vaccination was determined by enumeration of tubercle bacilli in lungs and spleen, histological examination of lungs, spleen and liver and estimation of cytokines in splenocyte culture supernatant after challenging them with *M. tuberculosis*.

Fig. 20: Protective effect of BCG in murine tuberculosis.



* P < 0.05

In lungs, at 8 weeks post challenge infection, control animals showed significantly higher CFU compared to all the other groups. At 12 weeks, there was no difference in CFU in all the groups of animals. In spleen, at 8 weeks, control animals showed significantly high CFU compared to animals in BCG, *M. terrae* and *M. fortutium* groups but not in animals in the *M. intracellulare* group. At 12 weeks, control animals showed significantly high CFU compared to all the other groups.

At 2 weeks, animals in the control and *M. intracellulare* groups showed minimal granulomatous response predominanted by macrophages. The other groups showed more granulomatous response, which was lymphocytic. At 8 weeks, there was no difference in granuloma formation as well as in cellular profile in all the groups. Elevated levels of TNF-α, IFN-γ and IL-4 were observed at 4 weeks post infection in splenocyte culture supernatant in control animals and at later weeks in *M. intracellulare* group. Animals in the other groups showed low or undetectable levels of all the cytokines.

Among the three NTM tested in mice, only *M. intracellulare* showed results similar to the non-immunized control animals in bacterial enumeration of spleen, histology and cytokine levels. Animals in *M. terrae* and *M. fortutium* groups showed results similar to the BCG immunized animals (Figure 20).

14. Identification of non tuberculous mycobacteria by HPLC

HPLC system (Waters) has been put to use for the routine identification of clinically significant non tuberculous mycobacteria (NTM). Mycolic acid analysis has been standardized using reference strains. 195 isolates have been analyzed so far and of these 46 of them were repeat excretors. The frequently found species were *M. avium complex, M. kansasii, M. simiae, M. fortutium, M. terrae* complex and *M. gordonae.* The chromatographic patterns are periodically sent to CDC, Atlanta for confirmation. Susceptibility testing for *M. avium* complex to clarithromycin and *M. simiae* to antituberculous drugs is being done. The study is in progress.

"β_2-microglobulin may be a specific marker for TB in HIV-TB patients; while neopterin, sTNF-RI and RII are non-specific markers."

15. Study of immune activation markers in patients with HIV and tuberculosis

Co-infection with tuberculosis and HIV synergistically leads to a heightened state of systemic immune activation. Some of the plasma activation markers that have been reported to be increased in HIV infection include neopterin, β_2-microglobulin, sTNF-RI and RII. The relationship between these markers and HIV and tuberculosis needs further delineation.

The aim of the present study was to estimate plasma levels of soluble immune activation markers (neopterin, β_2-microglobulin, sTNF-RI and RII) in HIV infected patients with and without tuberculosis and determine their relationship to prognosis.

The study population comprised of four groups of individuals; (i) 42 individuals with HIV-TB, (ii) 20 HIV patients without tuberculosis, (iii) 20 HIV negative tuberculosis patients and (iv) 38 healthy controls. While tuberculosis patients were started on anti-TB treatment and assessed again on completion of anti-TB treatment, none of the HIV patients received antiretroviral treatment at the time of the study. Plasma levels of activation markers were determined by capture ELISA using immunoassay kits (Biosource International, Belgium).

Plasma levels of neopterin, β_2-microglobulin, sTNF-RI and RII were significantly elevated in HIV patients and TB patients as compared to healthy controls. Patients with HIV-TB had significantly higher levels of these markers as compared to those with HIV alone. While plasma levels of all activation markers declined during anti-TB treatment in HIV-TB patients, only β_2-microglobulin reached comparable levels as in HIV patients without TB. Post-treatment levels of neopterin, sTNF-RI and RII were significantly higher in HIV-TB patients than in HIV patients without tuberculosis.

16. Neopterin as a surrogate marker of response to treatment in patients with HIV and tuberculosis

The objective of the present study was to evaluate serum neopterin as a surrogate marker of response to treatment in patients with HIV and tuberculosis. Serum neopterin was measured by HPLC at the beginning, during and at the end of treatment in 25 HIV positive and 10 HIV negative tuberculosis patients. Ten asymptomatic HIV positive individuals and 10 healthy controls were also included.

The median levels of serum neopterin with 25th and 75th percentiles are shown in Table 7. Patients with HIV and tuberculosis have been divided into two groups based on their initial CD4 count. Serum neopterin level was significantly higher in patients with active tuberculosis compared to controls, regardless of HIV status. The level was highest in patients with HIV and tuberculosis with

CD4 count < 200/mm^3. Neopterin levels in asymptomatic HIV positive subjects were significantly higher than in healthy subjects but lower than in patients with HIV and tuberculosis. The effect of anti-TB treatment showed a gradual decrease in neopterin levels but the median level in HIV patients with tuberculosis at the end of treatment was significantly higher than that of HIV-negative tuberculosis patients (39 vs 13 nmol/l, p <0.05).

Table 7: Serum neopterin levels in study population

Subject Category	Number of Subjects	Serum neopterin (nmol/l) (25th – 75th percentile)
HIV+ TB+ CD4 ≥ 200	10	38 (25-48.5)
HIV+ TB+ CD4 < 200	15	91 (55-125)
Pulmonary TB, HIV-	10	29 (19-42)
Asymptomatic HIV+	10	13.5 (12-14.5)
Healthy Subjects	10	7.4 (6-8)

"Serum neopterin levels are indicative of immune activation and are elevated in asymptomatic individuals with HIV infection as well as in those with tuberculosis. Neopterin levels can be combined with CD4 counts to improve prognostic value."

17. Lymphocyte apoptosis in patients with HIV infection and disease

HIV infection is associated with a progressive decrease in CD4+ T lymphocytes resulting in immunodeficiency and increased susceptibility to opportunistic infection and malignancies.

The present study was carried out to evaluate the extent of lymphocyte apoptosis by studying DNA fragmentation. The study subjects comprised of patients with HIV (n=10), HIV and tuberculosis (n=10), tuberculosis (n=10) and normal healthy volunteers as controls (n=10). Peripheral blood mononuclear cells (PBMCs) were isolated from 10 ml of heparinised venous blood and cultured with Phyto Haemagluttin (PHA) for three days. The genomic DNA was extracted from freshly isolated as well as cultured PBMCs. The presence of any fragmented DNA was detected by electrophoretic separation on agarose gel. Results are shown in Table 8.

Table 8: Lymphocyte apoptosis in various categories

Samples	No. of samples with observed apoptosis			
	HIV (n=10)	HIV-TB (n=10)	TB (n=10)	Volunteers (n=10)
Freshly isolated PBMCs	Nil	6	Nil	Nil
Cultured PMBCs (unstimulated)	2	7	1	Nil
PHA stimulated PBMCs	4	10	3	1

"HIV-infected patients with tuberculosis showed increased apoptosis of PBMCs."

18. Lymphocyte profile in HIV infected individuals

The aims of the study were to: 1) quantitate the lymphocyte subset numbers in the South Indian normal population as our laboratory reference value, 2) analyze the distribution of lymphocyte subsets in HIV positive subjects by immunophenotyping using flow cytometry and 3) compare the flow cytometric method of CD4 enumeration with alternative methods available for CD4 counting.

Immunophenotyping was carried out in the peripheral blood from HIV sero-positive individuals (n=225) and normal healthy volunteers (n=104) by flow cytometry using FACSORT.

Haematological parameters such as total WBC count, RBC count, haemoglobin, platelets, leucocyte percentages and numbers were recorded. The percentage of B-cells (CD19), total T-cells (CD3), Helper T-cells (CD4), Suppressor T-cells (CD8) and Natural Killer cells (CD16+56) were measured by flowcytometry. The absolute subset counts were calculated as the product of the total WBC, percent lymphocytes and percent subset cells.

The mean values and range of total WBC, as well as lymphocyte percentages and absolute counts are shown in Table 9. The mean and range of reference values calculated in Becton Dickinson multicentric study is also given.

The mean WBC counts and lymphocyte counts are significantly higher than the western reference values, while lymphocyte percentage did not differ significantly.

Table 9: Comparison of South Indian normal values with reference values – Lymphocytes

	WBC per mm^3	LYM%	LYM# per mm^3
Mean	7717	32.1	2317
S.D	1816	8.0	691
Median	7550	31.7	2200
95% Range	7368 - 8066	30.6 - 33.6	2184 - 2450
Reference			
Mean	5660	29.8	1687
Range	3500 - 9400	19 - 43	1075 - 2434
	S	NS	S

WBC White blood corpuscles
LYM% Lymphocyte percentage
LYM# Lymphocyte number
NS Not significant
S Significant

The lymphocyte subset absolute numbers and CD4:CD8 ratio are shown in Table 10, and compared with the reference values. Except for CD16+56 positive NK cells, the mean percentages of all the other subsets, such as CD3, CD4, CD8 and CD19 are significantly higher than that of western reference values, while CD16+56 are similar.

Table 10: Comparison of South Indian normal values with reference values – Subsets

	CD 19	CD 3	CD 4	CD 8	CD 16+56	CD 4 / CD 8
Mean	304	1592	833	682	270	1.4
S.D	203	592	355	351	176	0.6
Median	253	1516	765	603	224	1.3
95% Range	265 - 343	1478 - 1706	764 - 901	615 - 749	236 - 304	1.26 - 1.48
Reference						
Mean	230	1200	785	440	290	1.9
95% Range	100 - 430	720 - 2330	430 - 1760	170 - 1050	90 - 430	0.9 - 3.4
	S	S	S	S	NS	S

NS Not significant
S Significant

Since the mean CD4 and CD8 counts in our normal subjects are higher than the western reference values, the CD4/CD8 ratio is significantly lower than western reference value.

The CD4 and CD8 counts among the males (N=50) and females (N=54) were separately analysed. The difference between the sexes was not significant.

Alternate technologies for CD4 measurement were carried out. The CD4 levels in blood (N=115) have also been measured using TRAX ELISA and compared with flow-cytometry. Other surrogate markers for CD4, which can be cost-effective, such as total lymphocyte count and CD4%, are also being analysed.

The phenotyping results available for the HIV infected individuals are also being analysed and correlated with other laboratory parameters, such as viral load.

"The mean WBC and lymphocyte counts are significantly higher in South Indian population compared to western reference values, while the CD4/CD8 ratio is significantly lower."

19. Malabsorption of rifampicin and isoniazid in HIV infected patients with and without tuberculosis

Malabsorption of drugs from the gastrointestinal tract due to HIV enteropathy and concurrent infections could lower the bioavailability of anti-tuberculosis drugs in HIV infected individuals. This can have implications for tuberculosis treatment. Our aim was to study the absorption of

rifampicin(RMP), isoniazid (INH) and D-xylose in Indian subjects with HIV & diarrhoe and HIV & TB.

The participants comprised of 23 HIV-seronegative pulmonary TB patients (Group 1), 40 patients with advanced HIV infection and diarrhoea (Group 2) and 26 patients with HIV & TB (Group 3). Rifampicin (450 mg) and INH (300 mg) were administered orally followed by D-xylose (5 g). Urine was collected upto 8 hours. D-xylose absorption test was performed in 10 healthy volunteers (mean age 38.0 years and mean body weight 65.4 kg).

The concentrations of RMP and its primary metabolite, desacetyl RMP (DRMP) were measured by a HPLC method developed in our laboratory. The concentrations of INH and its metabolite, acetyl INH (AcINH) and that of D-xylose were measured by spectrophotometric methods. The values were expressed as percent dose of RMP (RMP & DRMP), INH (INH & AcINH) and D-xylose excreted in urine. All estimations were undertaken after coding the samples. The mean percent dose of xylose excreted in urine in TB patients was 30.25 which was not significantly different from that of the healthy volunteers (30.04). Hence, for further comparison with respect to drug levels, Group 1 was treated as the control group against which groups 2 and 3 were compared. The mean percent dose of xylose in groups 2 and 3 were 16.98 and 20.87 respectively, both being significantly lower than that of group 1 and healthy volunteers ($p < 0.001$) (Figure 21).

"A significant degree of malabsorption was observed among patients with advanced HIV infection, with and without diarrhoea."

A significant reduction in the percent dose of RMP & DRMP excreted was observed in groups 2 and 3 when compared to group 1, the values being 7.35, 6.60 and 10.06 respectively ($p < 0.01$). The excretion of RMP & DRMP was reduced by 27 and 34 % in groups 2 and 3 respectively (Figure 22).

The mean percent doses of INH & AcINH excreted in groups 1, 2 and 3 were 49.6, 37.9 and 38.3 respectively. A significant decrease in the excretion of INH and AcINH was observed in groups 2 and 3 compared to group 1 ($p < 0.05$) and the reduction in the excretion of the drug in the former groups was 24 and 23 % respectively (Figure 23). No significant differences with respect to urinary excretion of D-xylose, RMP and INH were observed between groups 2 and 3.

Fig. 21: Urinary levels of xylose in different study groups

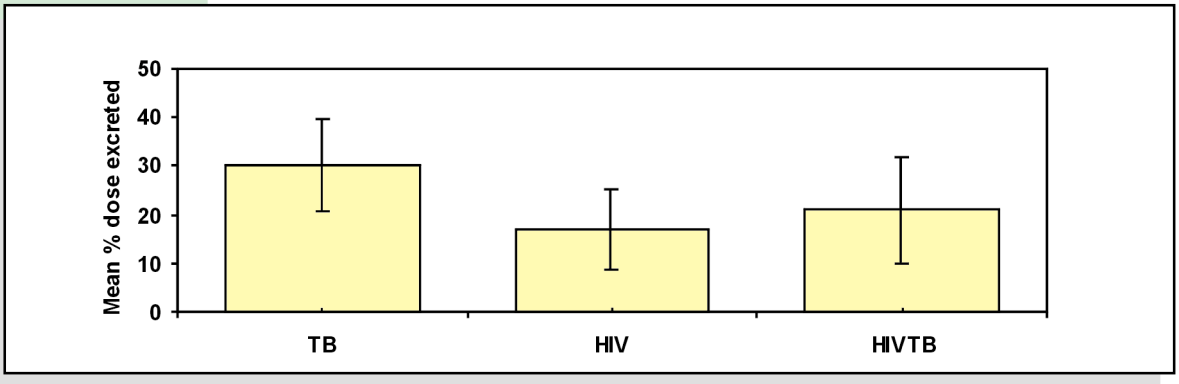


Fig. 22: Urinary levels of RMP and DRMP in different study groups

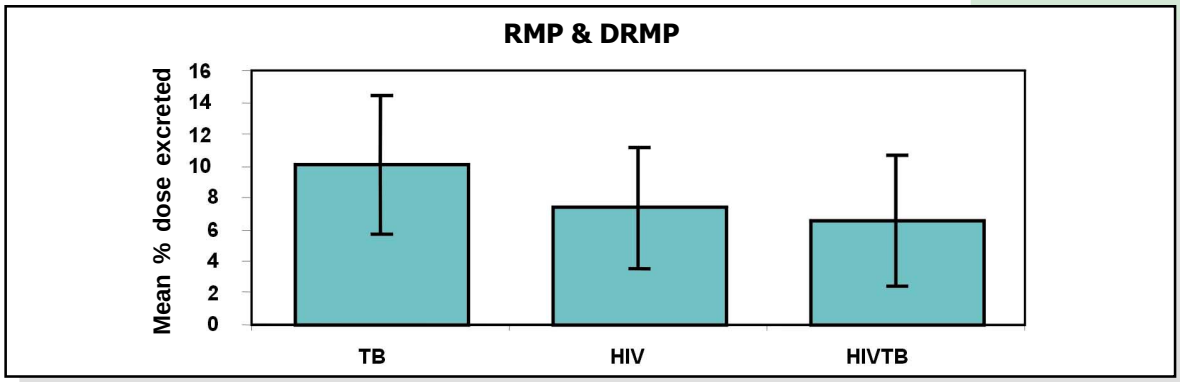
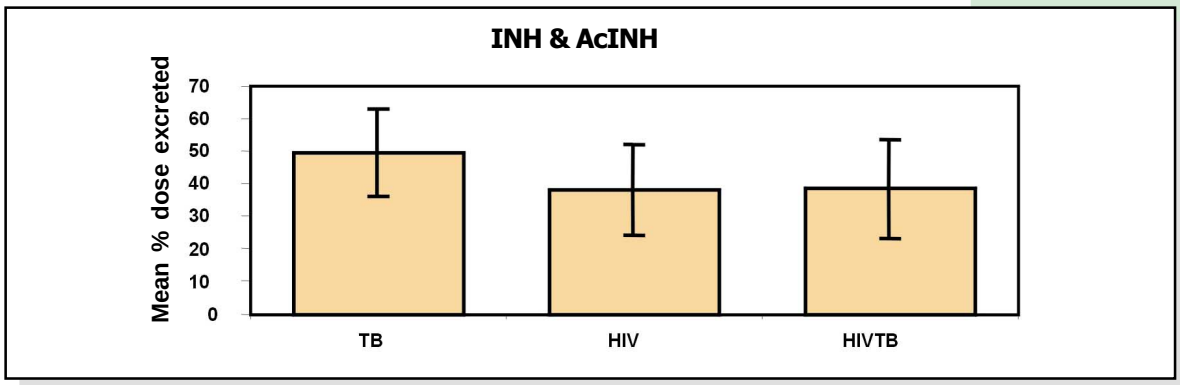


Fig. 23: Urinary levels of INH and AcINH in different study groups



A significant positive correlation existed between percentage dose of xylose and that of RMP and INH in urine, (r= 0.55 and 0.49 respectively, p < 0.001). However, the CD4 lymphocyte counts correlated significantly only with percentage dose of RMP (r= 0.38, p <0.01). Also the correlation between percent dose of RMP and INH was significant (r=0.34, p <0.01).

The data obtained in this study clearly demonstrate that the urinary excretion of both RMP and INH was significantly lower in both the HIV groups when compared with the TB group. These findings are supported by a significant decrease in xylose absorption as well.

20. Pharmacokinetics of anti-tuberculosis drugs in HIV infected patients with and without tuberculosis

Although most HIV infected patients with tuberculosis, respond well to rifampicin based antimycobacterial drug regimens, recent reports suggest that malabsorption of antimycobacterial drugs occurs in selected HIV infected patients. In order to ascertain the clinical significance of these findings, we undertook a pharmacokinetic study to assess the peak concentration and exposure of RMP and INH and to correlate these parameters with the response to treatment with RMP containing regimens in patients with HIV and HIV and tuberculosis.

A study on the pharmacokinetics of RMP, INH, pyrazinamide (PZA) and ethambutol (EMB) has been undertaken in HIV infected Indian subjects. A total

of 13 HIV negative patients with smear positive pulmonary tuberculosis, 13 with HIV/ diarrhoea and 14 with HIV and tuberculosis are included in the study. Rifampicin (450mg), INH (600mg), PZA (1500mg) and EMB (1200mg) are administered orally. The plasma levels of RMP and INH at different time points and urinary levels of all drugs/metabolites are estimated. Pharmacokinetic variables based on the plasma estimates and the percentage dose of the drugs excreted based on urine estimates are calculated.

The study has been completed and analysis is in progress.

21. Standardization of a method for the estimation of ethambutol by column chromatography

"A simple method to quantify ethambutol in pharmaceutical preparations and urine without the interference of other anti-TB drugs has been standardized."

Formulations containing Ethambutol (EMB) along with other anti-TB drugs, have been processed for the estimation of EMB. Fifteen tablets with EMB alone (Ethambutol hydrochloride-single drug-200 mg, 400 mg and 800 mg of each), 4 tablets with RMP and INH , 6 tablets containing EMB and INH , 11 tablets containing RMP, EMB and INH and 26 tablets containing RMP, EMB, INH and PZA were processed by this method. The drug was also estimated in urine samples collected from patients.

The method involved extraction of EMB into an organic solvent, followed by basification and column chromatographic separation on Amberlite CG 50 (100-200 mesh) and elution with suitable eluants and estimation at a wavelength of 270 nm. The assay was linear from 25 to 400 µg/ml. The relative standard deviations of intra and inter day assays were lower than 5%. The amount of EMB present in various formulations could be easily determined by this method.

22. Role of antioxidants in patients with diabetes and tuberculosis

Anti-oxidant defenses are lowered in non-insulin dependent diabetes mellitus (NIDDM). This diminished anti-oxidant reserve may be due to competition for NADPH, which is a cofactor required to recycle the oxidized free radical scavenger back to its effective reduced form.

It was aimed to investigate the possible changes in concentrations of the free radical scavengers such as superoxide dismutase (SOD), glutathione peroxidase and glutathione reductase and to investigate the antioxidant levels in all the 3 groups of patients namely, diabetes (DM), tuberculosis (TB) and diabetes/tuberculosis (DM/TB) and also to evaluate the lipid peroxidation in plasma in terms of malondialdehyde (MDA) released. The lipid peroxide (LPO) levels are markedly elevated (Table 11) in all the three groups of patients when compared with healthy controls. A statistically significant difference was observed between the controls and patient groups ($p<0.005$) and there was a significant difference even when the comparison was made among the groups ($p<0.001$).

Table 11: Antioxidants in various categories

Groups	Mean ± Standard Deviation	
	LPO nmols/ml	SOD Units/mgHb
Controls	0.628 ± 0.148	0.028 ± 0.004
Diabetes	1.557 ± 0.542	0.042 ± 0.005
Tuberculosis	3.542 ± 0.547	0.050 ± 0.012
Diabetes & Tuberculosis	2.457 ± 0.333	0.033 ± 0.009

The SOD activity was increased in diabetes ($p < 0.001$) compared to controls and also in tuberculosis ($p < 0.003$). But in DM/TB patients the change in SOD activity did not attain significant difference. Apart from controls, there was a significant difference observed among the study groups (Group ii, iii, & iv). The p values were <0.002 and <0.01 for diabetes & DM/TB and TB & DM/TB respectively.

"Elevated levels of superoxide dismutase were observed in patients with diabetes when compared to those with TB & diabetes. There was marked elevation of LPO in TB compared to diabetes and diabetes & TB."

23. Absorption of rifampicin and xylose in leprosy patients in the presence of clofazimine and dapsone

The bioavailability of rifampicin(RMP) in the presence of other anti-leprosy drugs namely dapsone and clofazimine was undertaken in leprosy patients. D-xylose absorption test to assess the absorptive capacity of the intestines was also carried out.

A total of 8 patients with leprosy who were on treatment in GREMALTES, Chennai, were selected for the study. Rifampicin (600 mg), Dapsone (100mg) and Clofazimine (300 mg) were administered on an empty stomach. Two hours later, a uniform oral dose of D-xylose 5 g was administered. Urine upto 8 hours was collected and the levels of RMP & desacetyl rifampicin and xylose were estimated using standard procedures.

Table 12 gives the percentage dose of RMP and xylose excreted in urine during 0-8 hours. Both xylose and RMP excretion were much less in leprosy patients compared to that in patients with pulmonary tuberculosis(PTB).(This data is from the other study conducted simultaneously)

The mean percent xylose excreted in urine in tuberculosis patients was 30.2 which was not significantly different from that of the healthy volunteers (30.0). Hence, comparison was made with PTB patients.

"The absorption of RMP and xylose is significantly lower in leprosy patients."

Table 12: Levels of xylose & total RMP in PTB & Leprosy patients

Group	% Dose excreted (0-8 hours)	
	Xylose	Total RMP
PTB (n=23)	31.5 ± 4.3 } *	8.9 ± 3.9 } **
Leprosy (n=8)	12.4 ± 5.8	5.4 ± 2.6

* $P < 0.001$ ** $P < 0.009$

24. Characterization and purification of antigenic components of *M. tuberculosis*

The 27kDa (mpt 51) antigen was observed in our laboratory to be specifically recognized by TB sera in immunoblot. Further work was carried out with the following aims:

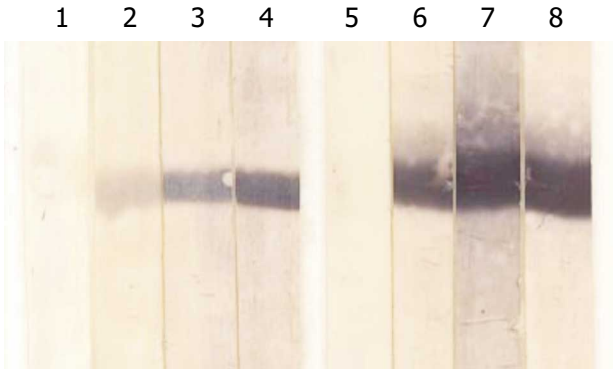
1. To purify and characterize the 27kDa antigen (native and recombinant)
2. To evaluate the diagnostic value of this antigen in various categories
3. To study the cellular response to this antigen in the host

Out of these objectives, the first one has been already achieved. Purification of the native antigen and over expression in *E. coli* and characterization of 27kDa (mpt 51) has been described in detail in previous years' annual reports. Here the results obtained on the diagnostic evaluation of these antigens are presented.

Antibody response in animals :

Polyclonal antibodies were raised against recombinant 27kDa protein in rabbits. There was an increase in the antibody titre after each immunization as shown by ELISA. A qualitative analysis of the produced polyclonal antibodies was also carried out by immunoblot experiments. When the pre and post-immunized rabbit sera were probed against recombinant 27kDa protein, prominent bands were visualized, confirming the recognition of the antigen by the host and immunogenicity of the antigen. In addition, when antisera produced against 27kDa were used to probe the whole culture filtrate, antibodies recognized the native 27kDa present in the culture filtrate along with the antigen 85 complex (Figure 24), with which the 27kDa shares homology.

Fig. 24: Blot Picture representing polyclonal antibody against 27kDa antigen



Antigen:
Lane 1 to 4: 27kDa
Lane 5 to 8: Culture filtrate

Blotted with :
Lane 1 and 5: Preimmune sera against 27kDa
Lane 2 and 6: I immunization against 27kDa
Lane 3 and 7: II immunization against 27kDa
Lane 4 and 8: III immunization against 27kDa

Antibody response in human tuberculosis :

As shown in Table 13, 100/175 smear and culture positive pulmonary TB sera (S+C+) and 3 of the 150 normal, healthy subject (NHS) were positive for IgG antibodies. Thus the test showed a sensitivity of 57% and specificity of 98%. The positivity for IgA and M antibodies among the S+C+ was 47% and 13% respectively. When positivity for IgG and/or IgA was considered, it was seen that 24 additional S+C+ sera were positive for IgA, increasing the sensitivity of the test from 57% (100/175) to 71% (124/175). There were 5 additional positives in the NHS group, thus the specificity reduced to 95%. IgM positivity along with IgG and A, altered the sensitivity to 72% (126/175), with a specificity of 93%.

"27 kDa antigen may aid in improving the diagnosis of TB when used along with other antigens."

Table 13: ELISA positivity for TB and NHS against 27kDa antigen

CATEGORY	TOTAL NO	Ig G		Ig G + Ig A		Ig G + Ig A + Ig M	
		NO.	% + ve	NO.	% + ve	NO.	% + ve
TB	175	100	57	124	71	126	72
CONTROL	150	3	2	8	5	11	7

SENSITIVITY (for Ig G+Ig A+Ig M) 72%
SPECIFICITY (for Ig G+Ig A+Ig M) 93%

Generally it is believed that recombinant proteins do have a limited sensitivity when used for immunodiagnosis. Many recombinant proteins of *M. tuberculosis* were shown to be poorly recognized by the immune response initially elicited by the native antigens. The limited recognition of individual cloned proteins by patient antibodies may be a consequence of their lack of important antigenic determinants and of post-translational modification. But the recombinant 27kDa was found to be very useful in providing better sensitivity for the immunodiagnosis of tuberculosis subjects. Hence this antigen can be used along with the existing panel of antigens to improve the diagnosis of tuberculosis.

25. Immune responses in tuberculous pleuritis

Tumor necrosis factor – alpha (TNF-α) in pleural fluids :

In our earlier reports on humoral immune response to various mycobacterial antigens in tuberculous pleuritis(TP), we showed distinct recognition of antigens in 30-40 kDa region of immunoblot. However no marked differences were observed in the levels of antimycobacterial antibodies by ELISA.

We also studied the role of TNF-α at the site of disease in TP patients. Concentrations of TNF-α were measured directly in blood (BL) and pleural fluid (PF) samples. *In vitro* levels of TNF-α were also compared by stimulating the peripheral blood mononuclear cells (PBMC) and pleural fluid mononuclear cells (PFMC) with various mycobacterial antigens like PPD, culture filtrate (CF) and heat killed MTB (MTB).

"TNF-α plays a role in regulation of T cell apoptosis in TB pleuritis."

Our results showed, a significant (P<0.05) increase in TNF-α levels in PF when compared to BL in TP patients. There was no change in TNF-α levels in BL and PF of non-TB controls (Figure 25).

In vitro levels of TNF-α were significantly increased in PFMC of TP patients after PPD and MTB stimulation (P<0.05) suggesting the prior sensitization of PFMC by mycobacterial components. However in PBMC, there were no appreciable changes in TNF-α levels when stimulated with various mycobacterial antigens (Figure 26).

The increased *in vivo* TNF-α in pleuritis suggest the compartmentalization of TNF-α secreting cells at the site of disease and may contribute to the containment of infection by synergising with IFN-γ to activate infected macrophages or by regulation of T-cell apoptosis.

Fig. 25: Mean (±SEM) concentrations of TNF-α in plasma (BL) and pleural fluid (PF) of patients with tuberculous pleurisy (TB) and patients with effusions other than tuberculosis (Non-TB)

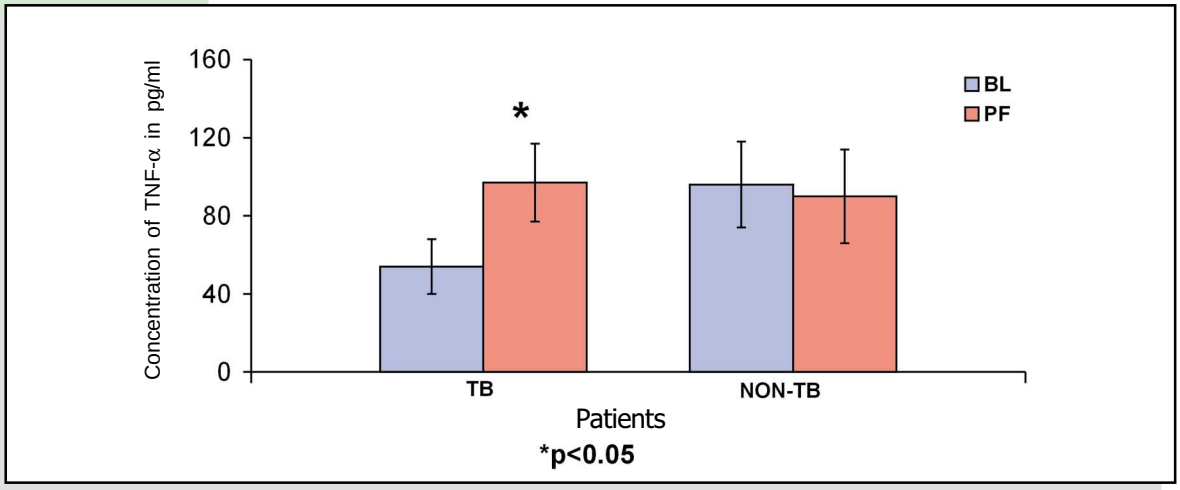
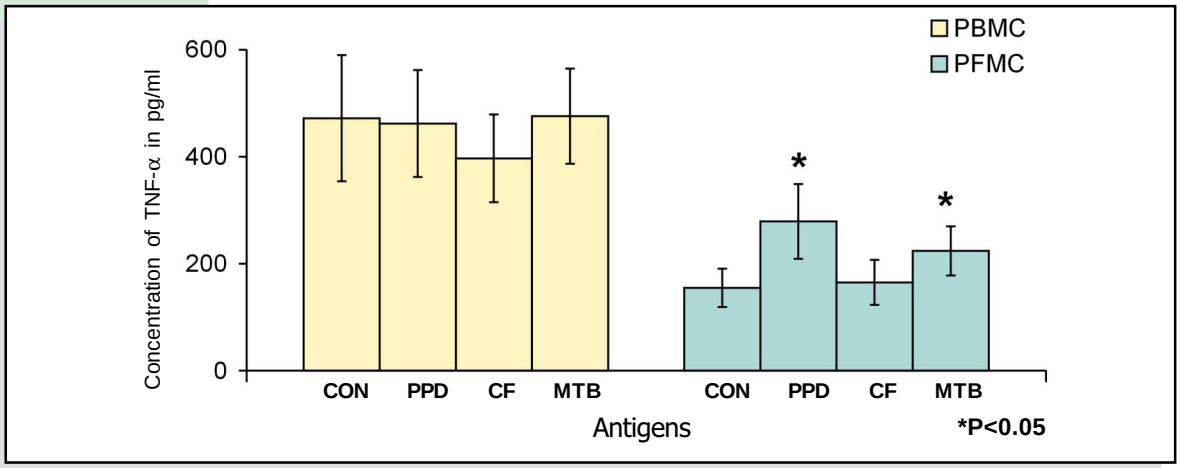


Fig. 26
In vitro production of TNF-α by peripheral blood mononuclear Cells (PBMC) and pleural fluid mononuclear cells (PFMC) by tuberculous pleuritis patients in the control (con) and in the PPD, Culture Filtrate (CF) and heat killed *M. tuberculosis* (MTB) stimulated conditions. The cytokine concentration was detected by ELISA in the supernatants after 48 hours of stimulation.



26. Non-HLA gene polymorphisms in pulmonarytuberculosis

Our earlier study revealed the association of *Bsm*I, *Apa*I and *Taq*I polymorphic variants of vitamin D receptor (VDR) genotypes with the differential susceptibility or resistance to pulmonary TB (PTB) patients. During the year, another VDR gene *Fok*I polymorphism was studied. An increased frequency of FF genotype (wild homozygotes) was observed in male PTB patients than male contacts (P=0.034; Odds ratio 2.76). A trend towards an increased frequency of ff genotype (mutant homozygotes) was observed in male contacts than male patients. No such difference in the genotype frequencies was observed among female patients and contacts. The study suggests that FF may be associated with susceptibility and ff with resistance to PTB in male subjects (Table 14). The *Bsm*I, *Apa*I, *Taq*I and *Fok*I polymorphisms of VDR gene and the differential susceptibility or resistance in males and females are presented in Table 15.

"Wild homozygotes (FF) may be associated with susceptibility and mutant homozygotes (ff) with resistance to PTB in male subjects."

Table 14: Genotype frequency of *Fok*I polymorphism of VDR gene

VDR *Fok*I Genotypes	Genotype frequency (%)					
	PTB (n=120)	Contacts (n=80) (n=74)	Male		Female	
			PTB (n=31)	Contacts (n=46)	PTB (n=49)	Contacts
FF	65.0	53.8	63.5*	38.7*	67.4	61.2
Ff	30.0	36.2	32.4	48.4	26.1	30.6
Ff	5.0	10.0	4.1	12.9	6.5	8.2

* FF: Male contacts vs Male PTB patients p=0.034; Odds Ratio: 2.76

Table 15: Association of VDR genotypes with susceptibility or resistance to pulmonary tuberculosis

VDR gene polymorphism	Susceptibility		Resistance	
	Male	Female	Male	Female
*Bsm*I	Bb (Heterozygotes)	–	BB (Wild Homozygotes)	–
*Apa*I	–	–	AA (Wild Homozygotes)	–
*Taq*I	TT (Wild Homozygotes)	tt (Mutant Homozygotes)	–	TT (Wild Homozygotes)
*Fok*I	FF (Wild Homozygotes)	–	–	–

"Homozygous for infrequent allele 2 (22 genotype) of BamHI polymorphism either alone or in combination with closely linked genes may be associated with bacteriological relapse."

27. *Bam*HI polymorphism of human cytochrome P_{450} gene, CYP2D6 in quiescent and relapse patients of pulmonary tuberculosis

*Bam*HI polymorphism of the human cytochrome P_{450} gene, CYP2D6, which encodes drug metabolizing enzyme, was studied to find out whether variant genotypes of this gene are associated with the susceptibility or resistance to bacteriological relapse of pulmonary tuberculosis after stopping treatment with short course chemotherapy of 6-8 months duration. No difference in the frequency of variant genotypes of *Bam*HI polymorphism of CYP2D6 gene was observed between pulmonary tuberculosis patients and control subjects. A trend towards an increased frequency of 22 genotype (homozygous for infrequent allele 2) was observed in bacteriological relapse patients (Table 16).

Table 16: Genotype frequency of *Bam*HI polymorphism of CYP2D6 gene in quiescent and relapse patients of pulmonary TB

Variant Genotypes	Genotype frequency (%)			
	Control (n=158)	PTB (n=154)	Quiescent PTB (n=50)	Relapse PTB (n=50)
11	29.1	25.3	30	22
12	43.7	49.4	52	48
22	27.2	25.3	18	30

28. Role of HLA-DR2, Mannose binding lectin (MBL), vitamin D receptor gene variants on immune functions in pulmonary tuberculosis

Our earlier studies revealed the association of HLA-DR2, functional mutant homozygotes of mannose binding lectin (MBL) and vitamin D receptor gene variants with susceptibility to pulmonary tuberculosis. It is known that the progression of tuberculosis is regulated/controlled almost entirely by the cell mediated immune response (CMI) of the host against pathogen. To understand the immunoregulatory role of these polymorphic gene variants on the immune mechanism of tuberculosis susceptibility, the present study has been planned and will be carried out in 60-70 pulmonary tuberculosis patients and 60-70 normal healthy volunteers.

During the year, immune functions such as phagocytosis with live *M. tuberculosis*, lymphocyte transformation test to *M. tuberculosis* culture filtrate antigen, apoptosis and cytokine estimation in culture supernatants of peripheral blood mononuclear cell stimulated with *M. tuberculosis* antigen were carried out in 30 pulmonary TB patients and 40 normal subjects.

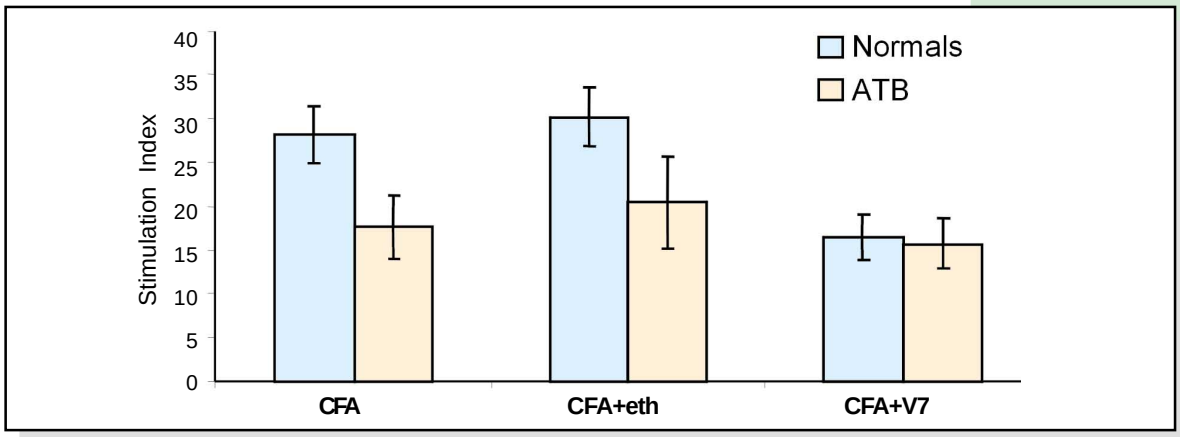
The results of the immune functions will be analysed separately as well as with vitamin D receptor, mannose binding lectin and HLA-DR gene variants to find out the role on immune functions.

Vitamin D receptor gene variants and Immune functions: Immunomodulatory effect of vitamin D_3 in pulmonary tuberculosis:

Vitamin D_3 (1,25 dihydroxy vitamin D) is an immunoregulatory hormone. Vitamin D acts through vitamin D receptor, nuclear hormone receptor. Vitamin D_3 has been shown to modulate immune functions.

Immune function studies with and without vitamin D_3 are being analysed. A decreased lymphocyte response to *M. tuberculosis* culture filtrate antigen (CFA) was observed in the presence of vitamin D_3(10^{-7}m) than the lymphocyte response without vitamin D_3 in normal healthy subjects ($P<0.05$). Such a decrease was not observed in pulmonary tuberculosis (Figure 27).

Fig.27 : Influence of Vit. D3 on the lymphoproliferative response to *M. tuberculosis* culture filtrate antigen in normal healthy subjects and pulmonary TB patients

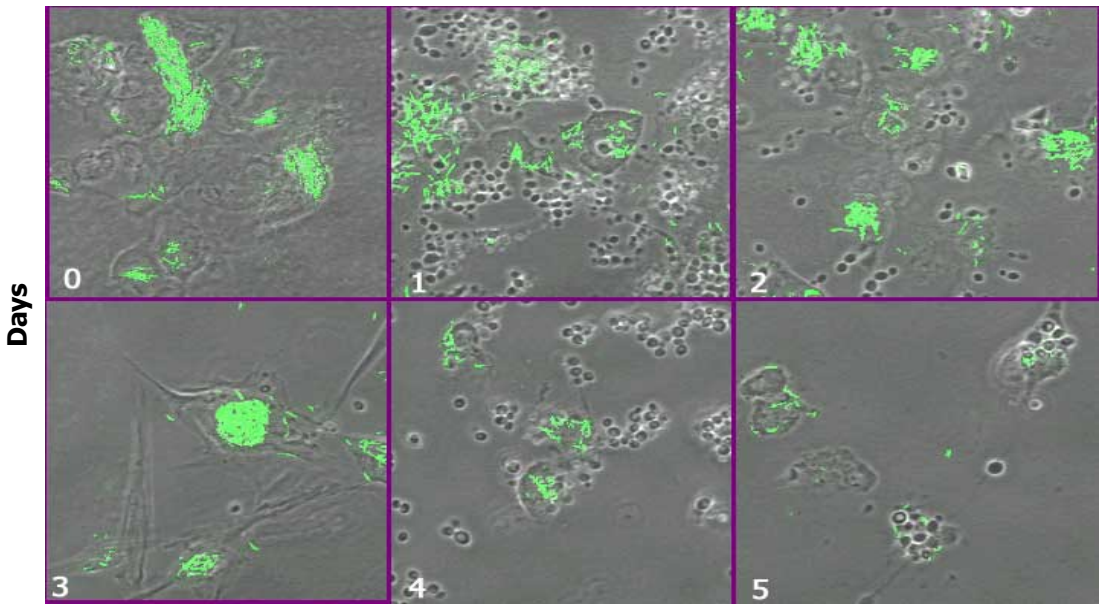


CFA – culture filtrate antigen; eth – ethanol; V7 - Vitamin D_3 (10^{-7}m)

29. Gene Regulation in Mycobacteria

Regulation of gene expression can be studied by analyzing the promoter region of the gene. One such promoter taken for analysis was a 400bp upstream region of *guaA* gene of *M. tuberculosis* H37Rv encoding the purine biosynthetic enzyme, guanosine mono phosphate synthetase. To learn if the *guaA* gene of *M. tuberculosis* is needed for its survival and whether it gets induced in the intracellular environment of macrophages, the 400bp putative promoter region of the *gua*A gene was PCR amplified and cloned upstream to green fluorescent protein gene in an *E coli*-mycobacterial shuttle vector and was named pKK TRC. The promoter of *guaA* gene of *M. tuberculosis* was induced in the intracellular environment of the macrophages (Figure 28) indicating that the *guaA* gene expression is necessary for the intracellular survival of *M. tuberculosis.*

Fig. 28: Human macrophages isolated from PBMCs infected with *M. tuberculosis* H37Rv harbouring pKKTRC



Promoter activity - observed for 5 days (0- 5 days)
Prolonged expression – importance in survival

30. Simultaneous infection with multiple strains of *M. tuberculosis* by restriction fragment length polymorphism analysis

A total of 543 patients with culture positive tuberculosis from the Model DOTS area were analysed by IS6110 and Direct Repeat Restriction Fragment Length Polymorphism (DR RFLP). The isolates showing different intensities of IS6110 band patterns were suspected of mixed infection and were further analysed by repeating RFLP on their alternate cultures and individual colonies.

The two isolates showed two and three distinct IS6110 RFLP (Figures 28a & 28b) patterns, respectively, with varied intensities of the bands indicating mixed

Fig. 28a

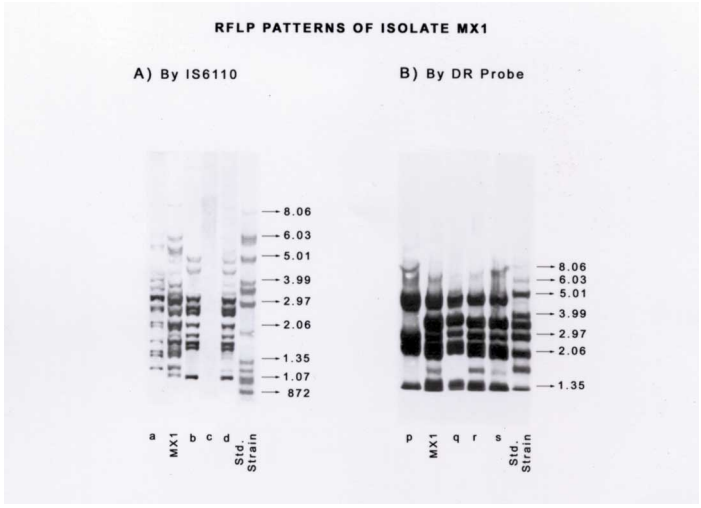
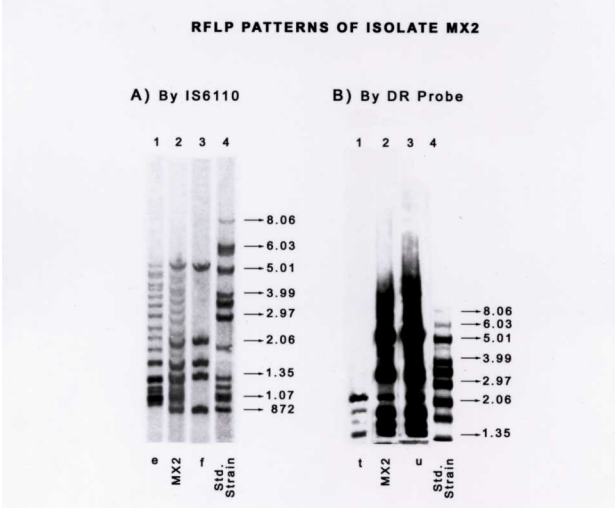


Fig. 28b



infection. The possibility of cross contamination was ruled out and the RFLP results on single colonies showed 2 to 3 different patterns for these isolates. Additional DR RFLP (Figure 28a & 28b) also confirmed infection with multiple strains. This study found evidence of co-infection with two or three different strains of *M. tuberculosis*, showing distinct RFLP pattern.

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

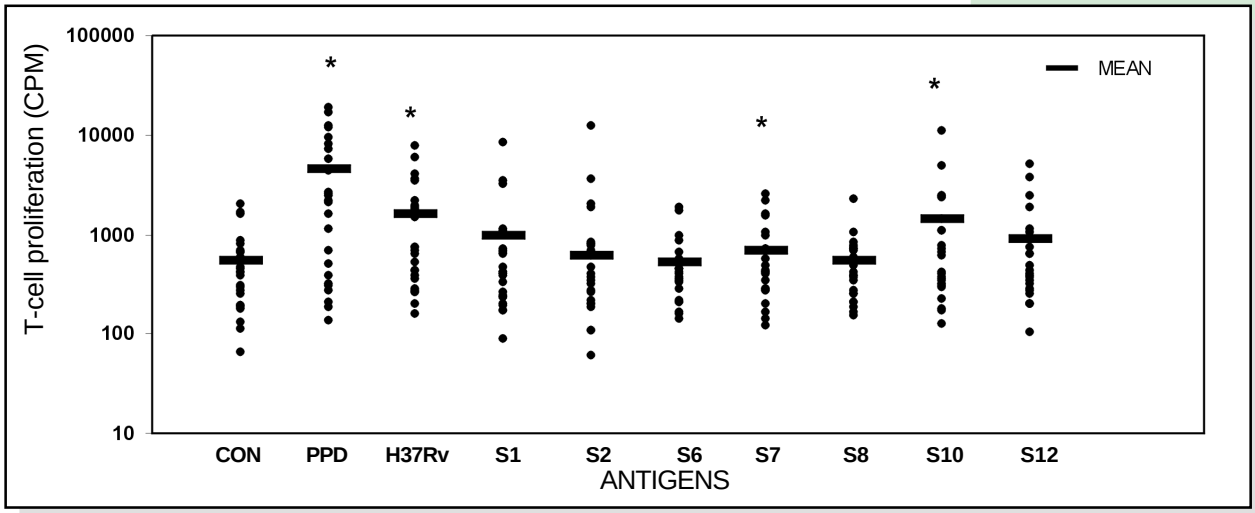
In our previous reports, we showed differential expression of many proteins by SDS-PAGE of the sonicate antigens prepared from the most prevalent strains of *M. tuberculosis* harbouring a single copy of IS6110.

The further immunological characterization of these sonicate antigens was done by studying their ability to induce protective immunity and comparing that with standard purified protein derivative (PPD) and heat killed *M.tuberculosis* H_{37}Rv antigens. Sonicate antigens from seven strains of *M. tuberculosis*, which showed differential protein profiles on SDS-PAGE were selected to study the *in vitro* T-cell proliferation and IFN-γ and IL-12 secretion in 25 normal healthy PPD positive subjects.

PPD and heat killed *M.tuberculosis* H_{37}Rv showed the maximum levels of T-cell proliferation (Figure 29) and IFN-γ secretion but low levels of IL-12 (Figures 30A and 30B). All sonicate antigens induced T-cell proliferation and IFN-γ secretion with good positive correlation (Figure 31). The antigens S10 and S12 showed higher levels of T-cell activation ($p<0.05$). All sonicate antigens showed uniform secretion of IL-12 but no significance was observed.

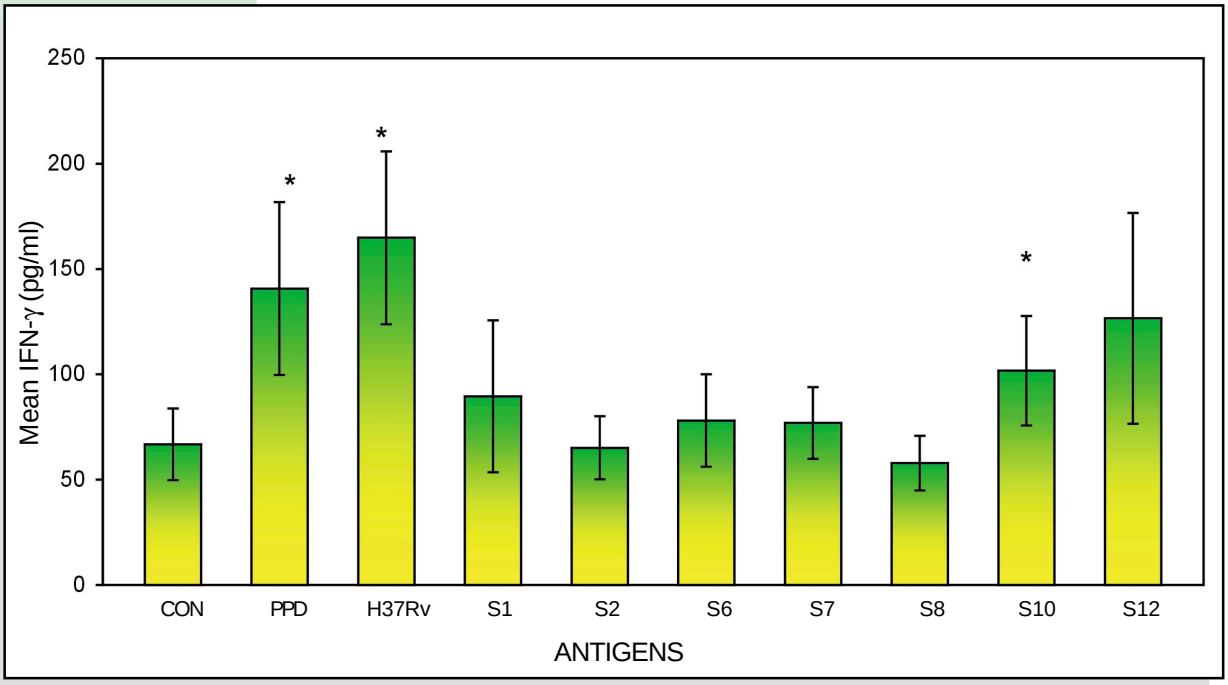
Our results suggest that sonicate antigens from most prevalent and recent strains of *M. tuberculosis* from clinical isolates have potential to induce T-cell activation and may have newer and specific antigens that could be further characterized for diagnostic purposes and vaccine development.

Fig.29: Lymphoproliferative response of 25 normal subjects to sonicate antigens from clinical isolates (S1, S2, S6, S7, S8, S10, and S12) of *M. tuberculosis* and standard antigens, PPD and H37Rv. Results are individual CPM values and mean. The statistical significance is shown as * = P < 0.05. (CPM = counts per minute)



Figs. 30: IFN-γ (A) and IL-12 (B) levels of normal laboratory volunteers induced by *M. tuberculosis* sonicate antigens from clinical isolates (S1, S2, S6, S7, S8, S10, S12) and standard antigens, PPD and H37Rv. Results are represented as mean ± SEM. The statistical significance is shown as *P < 0.05. (SEM = standard error of mean).

(A)



(B)

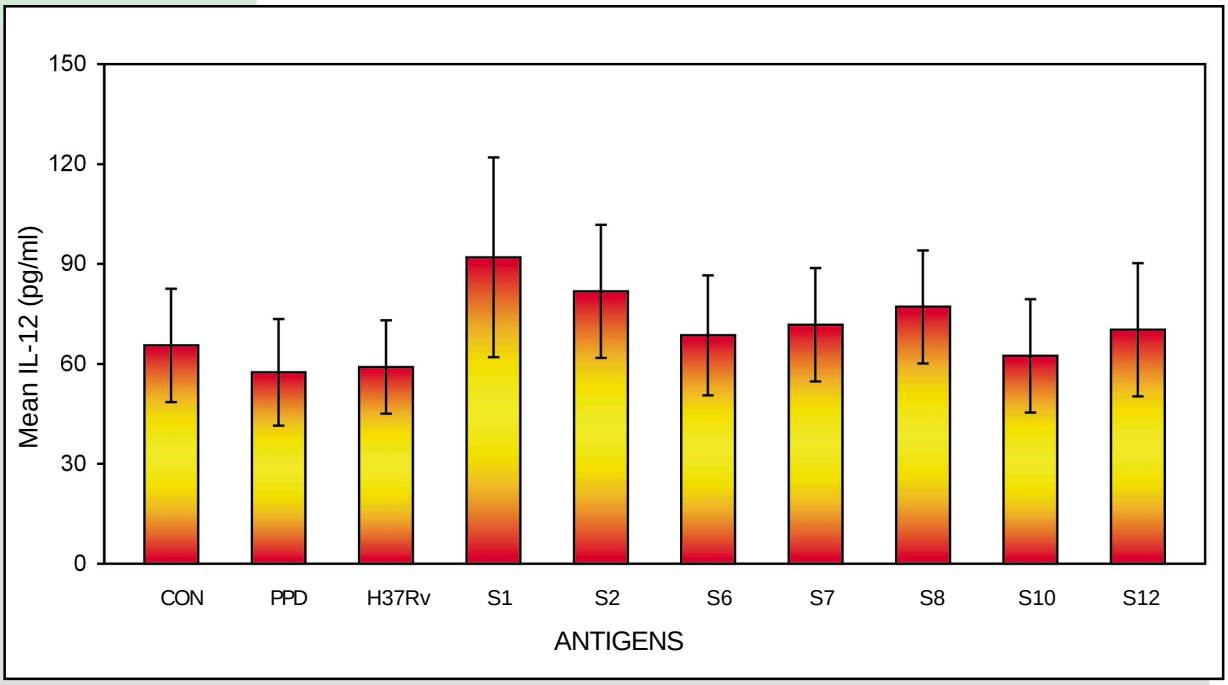
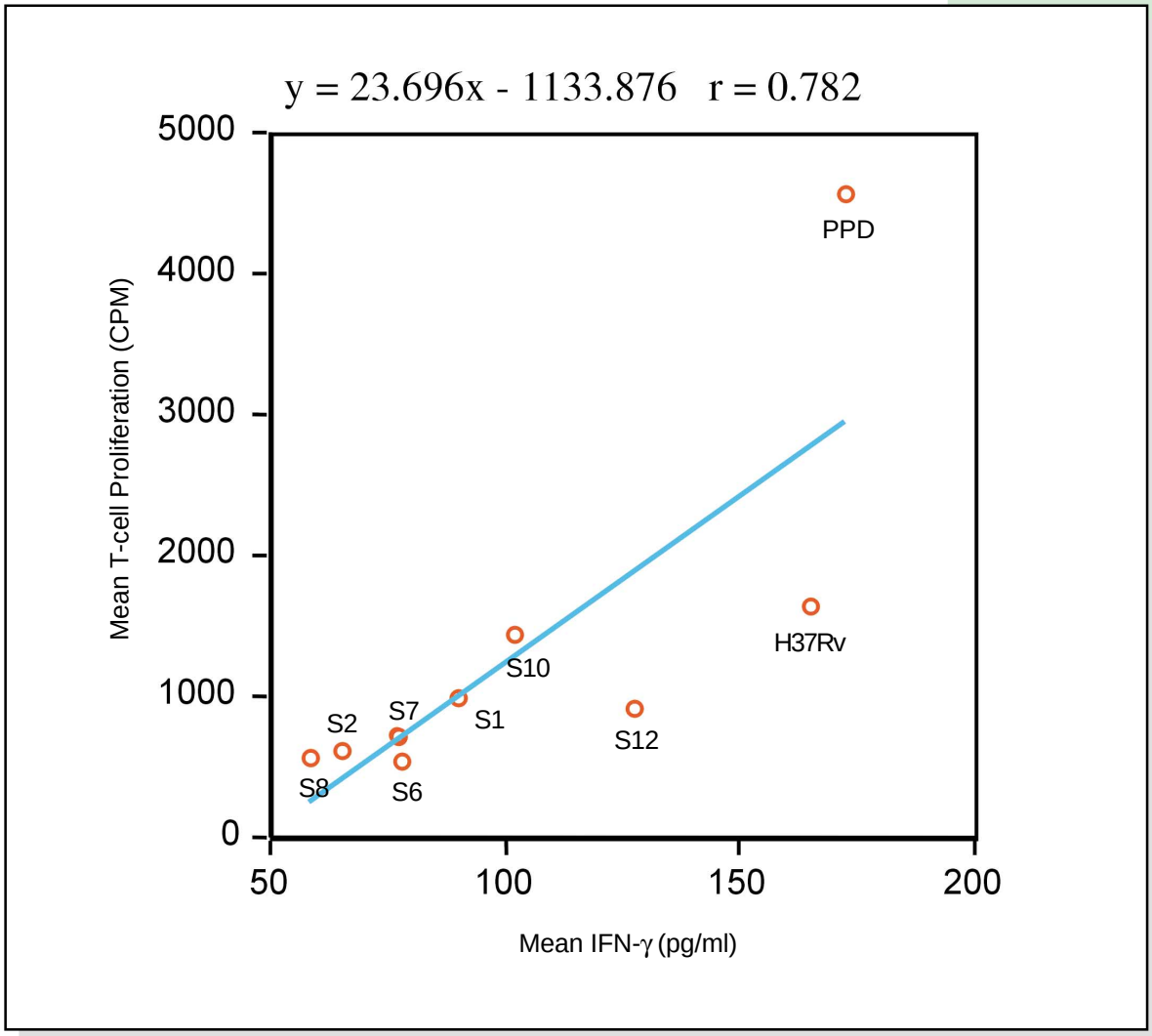


Fig. 31: Correlation of T-cell proliferation response and IFN-γ secretion of various sonicate antigens from clinical isolates of *M. tuberculosis* and standard antigens, PPD and H37Rv.



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

Guinea pigs that had been sensitised with a low dose of *M. tuberculosis* were treated with immune complexes (IC) containing *M. tuberculosis* and antibody in various proportions and then challenged with live *M. tuberculosis*. The animals were euthanised at various time points upto sixteen weeks after infection. Viable counts (VC) of *M. tuberculosis* in the spleen and histopathology of the spleen, liver and lung were carried out. During the period under review, this project was completed and the highlights of the results are given below:

The animals injected with antigen excess IC showed a persistence of tubercle bacilli upto sixteen weeks. The mean ± SE of log VC in the spleen is shown in Table 17.

"Prior treatment of guinea pigs with immune complexes in antigen excess leads to increased persistence of tubercle bacilli."

Table 17: Viable counts of bacilli in the spleen of guinea pigs.

GROUP	WEEKS AFTER INFECTION				
	1	4	8	12	16
CONTROL	1.53± 0.3	2.32± 0.4	0.94± 0.1	0.98± 0.1	1.47± 0.3
IC IN ANTIGEN EXCESS	2.65± 0.4	1.83± 0.1	1.95± 0.2	0.79± 0.1	0
IC IN ANTIBODY EXCESS	0	1.04± 0.1	1.1± 0.3	2.04± 0.3	0

When the histopathology results were analysed, liver and spleen showed no granuloma in any of the groups. But in the lung, granuloma was maximal in the control group and it was minimal in the group that had been pre-treated with immune complex in antigen excess.

33. Protective efficacy of recombinant BCG strains in guinea pig tuberculosis

"19 kDa antigen of mycobacteria may be immunosuppressive."

Recombinant strains of BCG over expressing esat-6, 19 kDa, 38 kDa, 85A, 85B and 85C antigens were injected into guinea pigs intradermally and nine weeks later, the animals were challenged with *M. tuberculosis* H37Rv ($1X10^2$ or $1X10^5$) subcutaneously. These animals were euthanised three and six weeks post infection and the viable counts of *M. tuberculosis* from the spleen and the histology of the liver and lung were assessed.

At six weeks, viable counts of tubercle bacilli obtained from the spleen indicated that while the protection afforded by rBCG expressing esat-6, 38 kDa and the 85 complex antigens ranged from 14-40%, there was no protection in the group vaccinated with 19kDa rBCG (Figure 32). Similarly, this was reflected in the histopathology of the lung, with maximal granuloma formation being seen in the PBS and 19 kDa groups (Figure 33).

Fig. 32: Viable counts of bacilli in the spleen of vaccinated guinea pigs.

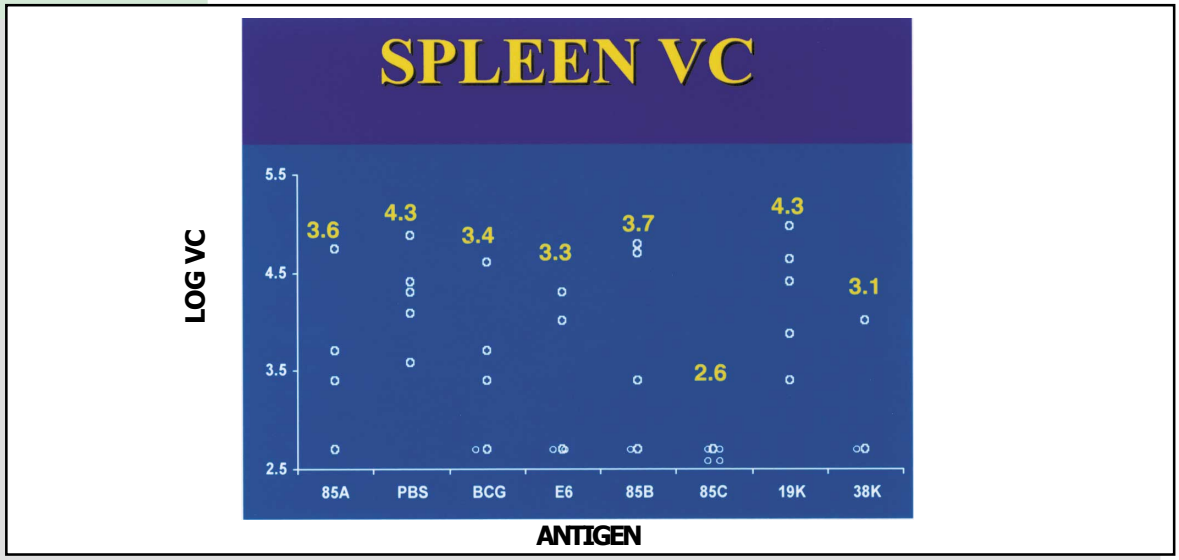
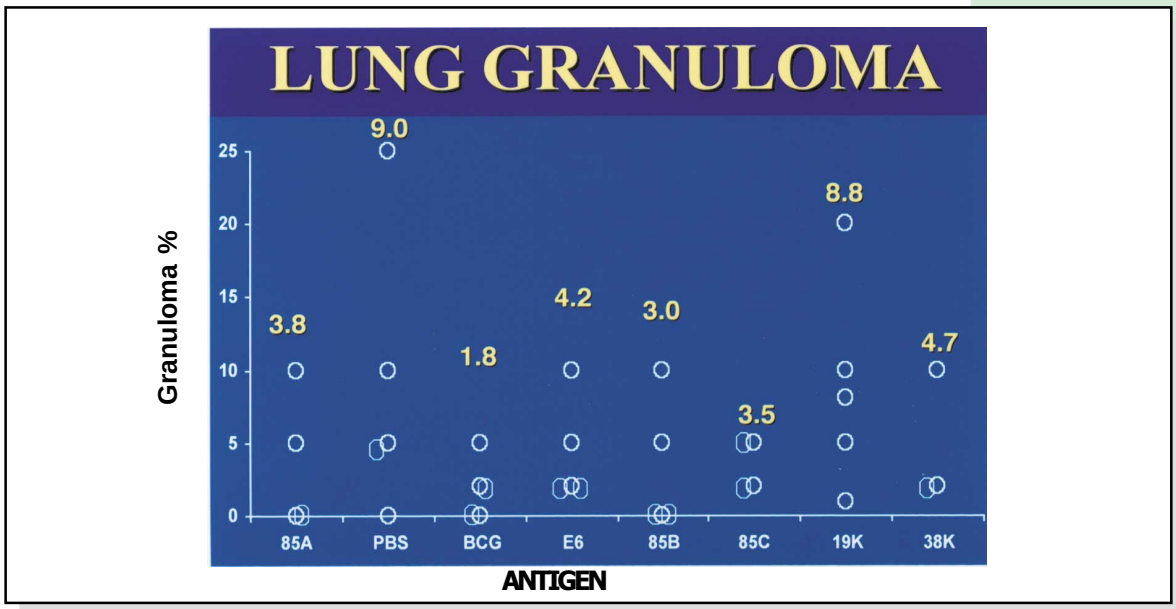


Fig. 33: Lung granuloma in vaccinated guinea pigs.

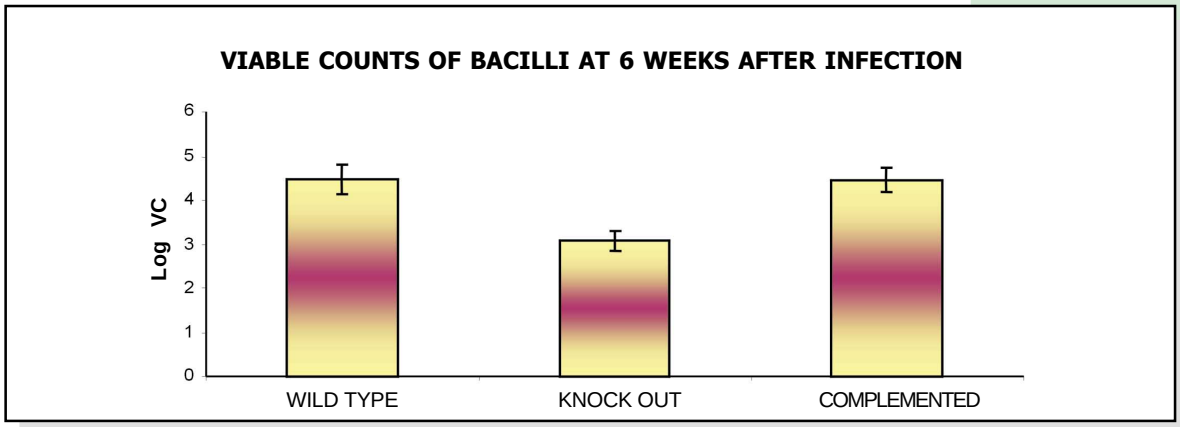


34. Infective potential of Tyrosine Phosphatase knock out strain of *M. tuberculosis*

The genome of *M. tuberculosis* has been shown to encode two tyrosine phosphatase genes (mptpA and mptpB) which have been implicated in the virulence of *M. tuberculosis*. *M. tuberculosis* strains in which these genes have been disrupted were constructed and used for infecting guinea pigs subcutaneously. The guinea pigs were euthanised three and six weeks post infection and the viable count of tubercle bacilli isolated from the spleen and lung, the histopathological changes in the liver and the lung from these animals were assessed.

The bacillary load in the spleen was similar in all the three groups of animals at three weeks after infection. However, at six weeks, infection with the mutant strain lacking mptpB gene resulted in a 70-fold reduction in the bacillary load compared to the animals infected with the parental strain. Upon reintroduction of the mptpB gene into the mutant strain, the complemented strain was able to establish infection and survive in guinea pigs at rates comparable to the parental strain (Figure 34).

Fig. 34: Viable counts of bacilli in the spleen of guinea pigs.



"The product(s) of the tyrosine phosphatase B gene of M. tuberculosis may be needed for survival of the bacilli in the host."

At 3 weeks post-infection, the livers from all the groups were similar. At six weeks post-infection, in the case of liver, granuloma was observed in three and five guinea pigs infected with the parental and complemented strains, respectively. Among the animals infected with mutant strain, only one animal exhibited granuloma.

It was found that at both 3 and 6 weeks, the lung sections from guinea pigs infected with the knock out strains showed significantly lesser granuloma compared to those from the animals infected with the wild type strain as shown in Table 18. Experiments using mptpA mutant strain are in progress.

Table 18: Lung granuloma in infected guinea pigs.

GROUP	3 WEEKS		6 WEEKS	
	Number of animals	Granuloma Index (%)	Number of animals	Granuloma Index (%)
M.tuberculosis (Wild type)	8	19.5 + 2.36	7	8.25 + 0.81
M.tuberculosis (Mptp B Knock out)	8	4.63 + 0.54	7	3.57 + 0.37
M.tuberculosis (Mptp B Complemented)	8	10.88 + 1.1	8	6.34 + 1.23

35. Protective efficacy of DNA vaccines in guinea pig tuberculosis

"DNA subunit vaccine containing superoxide dismutase confers partial protection against M. tuberculosis in the guinea pig."

To evaluate the protective efficacy of three candidate DNA vaccines, experiments were carried out in guinea pigs using two different doses of challenge with *M. tuberculosis* H37Rv ($1X10^5$ and $1X10^6$). In both the experiments, it was found that the candidate vaccine expressing the superoxide dismutase (SOD) antigen was the most efficient among the three candidates tested. With a challenge dose of $1X10^5$ CFU, the lung CFU of the animals immunized with the vaccine expressing SOD was found to be 1.69 log lower compared to the sham immunized animals. This was reflected in the granuloma content in the lung which was lower in the immunized group.

Whereas, the animals immunized with the vector backbone behaved similar to the saline treated group, the DNA vaccine expressing α-crystallin ranked second exhibiting a 0.5 log reduction in lung CFU. All these results were found to be statistically significant. However, none of the vaccines exhibited protection to the level provided by BCG.

The research activities of the Division of Statistics falls under three categories**.** The first category involves basic and applied statistical research in the development of new statistical models and methods. The important areas of research currently ongoing are survival analysis, HIV/AIDS modeling, artificial neural networks, competing risks, reliability, population genetics, and molecular computing. The second category pertains to providing scientific support to the controlled clinical trials and related laboratory experiments in study design, monitoring, quality control and evaluation. The third is the active involvement of the division in operational research studies and consultancy provided to biomedical and statistical scientists of other institutes.

1. Usage and acceptability of Indian system of Medicine & Homeopathy – A National Survey

The division of statistics of the centre in collaboration with IRMS New Delhi participated in the survey on "Usage and Acceptability of Indian System of Medicine & Homeopathy"(ISM & H) by the Ministry of Health & Family Welfare, Government of India in the Southern states. Six districts from 3 states (Nilgiris & Tirunelveli from Tamilnadu, Mehboobnagar & Nizamabad from Andhra Pradesh, Bellary & Dakshin Kannada from Karnataka) were covered. From each district, data were collected using separate schedules from a maximum of 12 hospitals, 24 dispensaries, 72 private practitioners, 240 patients, 50 villages/UFS blocks and 1000 households (Table 19). The information collected in the survey includes availability of facilities in the hospitals/ dispensaries under ISM&H in rural/ urban areas and also in Government/Non-Government sectors along with the extent of utilization of these services. Perception of the private practitioners, patients' attending the dispensaries, and households towards the ISM&H in health care was also collected in the survey as well as the factors coming in the way for utilization of these services.

Table 19: Number of units surveyed in each Districts.

Unit	Tirunelveli	Nilgiris	Bellary	Dakshin Kannada	Mehboob Nagar	Nizamabad
Hospital	12	5	5	2	-	1
Dispensaries	24	3	23	6	24	20
Pvt. Practitioners	25	-	-	4	8	12
Patients	43	30	230	60	240	206
Households	1000	928	750	929	780	960

Other Basic & Applied Statistical Research programs

Currently the department is involved in research works in the areas of survival analysis, HIV/AIDS modelling, artificial neural networks, competing risks, reliability, genetic modeling, health economics, LQAS and non-linear models.

PUBLICATIONS

Publications in	(i)	International journals	:	29
	(ii)	National journals	:	17
Accepted for publication in	(i)	International journals	:	6
	(ii)	National journals	:	5

Published

International

1. Gajalakshmi, P., Balasundaram, A., Venkatesan, P., Santhiya, T. S., Ramesh, A. Cytogenetic studies on spray patterns in South India. *Mutation Research, 2002,* **514**, 1-6.
2. Gajalakshmi, P., Mallick, P., Venkatesan, P., Santhiya, S. T., Ramesh, A. Studies on sister chromatid exchanges in peripheral lymphocytes of spray painters. *Journal of Environmental Pathology, Toxicology and Oncology, 2002,* **21**, 63-65.
3. Immanuel, C., Hemanthkumar, A. K., Gurumurthy, P., Venkatesan, P. Dose related pharmacokinetics of Ofloxacin in healthy volunteers. *International Journal of Tuberculosis and Lung Diseases, 2002*, **6**, 1-6.
4. Jayasankar, K., Shakila, H., Umapathy, K. C., Ramanathan, V. D. Biochemical and histochemical changes pertaining to active and healed cutaneous tuberculosis. *British Journal of Dermatology, 2002,* **146**, 977-982.
5. Kamalakannan, V., Geetha Ramachandran, Sujatha Narayanan, Vasan, S. K., Narayanan, P. R. Identification of a novel mycobacterial transcriptional regulator and its involvement in growth rate dependence and stringent control. *FEMS Microbiology Letters, 2002*, **209**, 261-266.
6. Kolappan, C., Gopi, P. G. Tobacco smoking and pulmonary tuberculosis. *Thorax, 2002,* **57**, 964-966.
7. Kuppusamy Balamurugan, Rama Rajaram, Thirumalachari Ramasami, Sujatha Narayanan. Chromium (III)-induced apoptosis of lymphocytes: Death decision by ROS and Src-family tyrosine kinase. *Free Radical Biology & Medicine, 2002*, **33**, 1622-1640.
8. Nirmala, R., Narayanan, P. R. Flowcytometry – A rapid tool to correlate functional activities of human peripheral blood lymphocytes with their corresponding phenotypes after *in vitro* stimulation. *BMC Immunology, 2002*, **3**, 1-7.
9. Nirmala, R., Mathew, R., Narayanan, P. R. Short communication : Reduced cytokine secretions by LAK cells of pulmonary tuberculosis patients in response to tumor targets *in vitro*. *Journal of Interferon Cytokine Research, 2002*, **22**, 617-620.

10. Paramasivan, C. N**.,** Venkataraman, P., Chandrasekaran,V, Bhat, S., Narayanan, P. R. Surveillance of drug resistance in tuberculosis in two districts of South India**.** *International Journal of Tuberculosis and Lung Diseases, 2002,* **6**, 479–484.

11. Rajeswari, R., Chandrasekaran, V., Suhadev, M., Sivasubramanian, S., Sudha, G., Renu, G. Factors associated with patients and health system delays in the diagnosis of tuberculosis, South India. *International Journal of Tuberculosis and Lung Diseases, 2002*, **6**, 789-795.

12. Ramalingam, B., Uma Devi, K. R., Soumya Swaminathan, Alamelu Raja. Isotype specific antibody response in childhood tuberculosis against purified 38kDa antigen of *Mycobacterium tuberculosis*. *Journal of Tropical Pediatrics, 2002,* **48**, 188 –189.

13. Santha, T., Garg, R., Frieden, T.R., Chandrasekaran, V., Subramani, R., Gopi, P. G., Selvakumar, N., Ganapathy, S., Charles, N., Rajamma, J., Narayanan, P. R. Risk factors associated with default, failure and death among tuberculosis patients treated in a DOTS Programme: Tiruvallur District, South India, 2000. *International Journal of Tuberculosis and Lung Diseases, 2002*, **6**, 780-788.

14. Selvakumar, N., Prabhakaran, E., Rehman, F., Frieden, T. R., Santha, T. Washing of new microscopic glass slides in dichromate solution does not influence sputum AFB smear results. *International Journal of Tuberculosis and Lung Diseases, 2002,* **6**, 270-272.

15. Selvakumar, N., Gomathi, M., Rehman, F., Narayanan, P. R. Evaluation of two-reagent cold staining method for detection of acid- fast bacilli. *International Journal of Tuberculosis and Lung Diseases, 2002*, **6**, 728-731.

16. Selvakumar, N., Fathima Rahman, Renu Garg, Rajasekaran, S., Nalini Sunder Mohan, Thygarajan, K., Sundaram, V., Santha, T., Thomas R. Frieden, Narayanan, P. R. Evaluation of the Phenol Ammonium Sulfate sedimentation smear microscopy method for diagnosis of pulmonary Tuberculosis. *Journal of Clinical Microbiology, 2002*, **40**, 3017-3020.

17. Selvakumar, N., Fathima Rahman, Rajasekaran, S., Narayanan, P. R., Thomas R Frieden. Inefficiency of 0.3% Carbol Fuchsin in Ziehl-Neelsen staining for detecting acid-fast bacilli. *Journal of Clinical Microbiology, 2002,* **40**, 3041-3043.

18. Selvaraj, R., Gopal, G., Raja, A., Kumaraswami, V. Pattern recognition technique in immunological antigenic tests to identify *Mycobacterium tuberculosis* infection. *Tuberculosis, 2002,* **82**, 261-266.

19. Shenoy, R. K., Suma, T. K., John, A., Arun, S. R., Kumaraswami, V., Fleckenstein, L. L., NA-Bangchang, K. The pharmacokinetics, safety and tolerability of the co-administration of diethylcarbamazine and albendazole. *Annals of Tropical Medicine & Parasitology, 2002*, **96**, 603–614.

20. Suma, T. K., Shenoy, R. K., Kumarswami, V. Efficacy and sustainability of a footcare programme in preventing acute attacks of adenolymphangitis in Brugian filariasis. *Tropical Medicine and International Health, 2002*, **7**, 763-766.

21. Sujatha Narayanan, Sulochana Das, Lalitha Hari, Vijaya Bhaskar Rao, D., Renu Garg, Thomas R. Frieden, Narayanan, P.R. Molecular Epidemiology of Tuberculosis in a rural area of high prevalence in South India. Implications for disease control and prevention. *Journal of Clinical Microbiology, 2002,* **40**, 4785-4788.

22. Beena E Thomas, Sudha Ganapathy, Jawahar, M. S. Responding to health emergencies: People oriented strategies from India. *Social Development Issues, 2002,* **24,** 40-44.

23. Laura O' Bryan, Paula Pinkston, Kumaraswami, V., Vijayan, V., Gordon Yenokida, Helene F Rosenberg, Ronald Crystal, Eric A Ottesen, Thomas B Nutman. Localized eosinophil degranulation mediates disease in tropical pulmonary eosinophilia. *Infection and Immunity, 2003,* **71,** 1337-1342.

24. Uma Devi, K. R., Ramalingam, B., Alamelu Raja. Antibody response to *Mycobacterium tuberculosis* 30 and 16 kDa antigens in pulmonary tuberculosis with human immunodeficiency virus coinfection. *Diagnostic Microbiology and Infectious Disease, 2003,* **46,** 205-209.

25. Paramasivan, C. N., Venkataraman, P., Vasanthan, J. S., Rahman, F., Narayanan, P. R. Quality assurance studies in eight state tuberculosis laboratories in India. *International Journal of Tuberculosis and Lung Diseases, 2003*, **7,** 522-527.

26. Santha, T., Garg Renu, Frieden, T. R., Subramani, R., Gopi, P. G., Chandrasekaran, V., Selvakumar, N., Thomas, A., Rajeswari, R., Balasubramanian, R., Kolappan, C., Narayanan, P. R. Are community surveys to detect tuberculosis in high prevalence areas useful? Results of a comparative study from Tiruvallur District, South India. *International Journal of Tuberculosis and Lung Diseases, 2003,* **7**, 258-265.

27. Mani, C., Selvakumar, N., Kumar, V., Narayanan, S., Narayanan, P. R. Comparison of DNA sequencing, PCR-SSCP Pha-B assays with indirect sensitivity testing for detection of rifampicin resistance in *Mycobacterium tuberculosis*. *International Journal of Tuberculosis and Lung Diseases, 2003,* **7,** 652-659.

28. Ramalingam, B., Uma Devi, K. R., Alamelu Raja. Isotype- specific anti-38 and 27-kDa (mpt51) response in pulmonary tuberculosis with human immunodeficiency virus coinfection. *Scandinavian Journal of Infectious Diseases, 2003,* **35**, 234-239.

29. Selvakumar, N., Dakshayani Govindan, Naik Ashok Chand, Thomas R Frieden, Narayanan, P. R. Processing of sputum samples in a refrigerated centrifuge does not increase the rate of isolation of *Mycobacterium tuberculosis. Journal of Clinical Microbiology, 2003*, **41**, 469-471.

National

1. Anbupalam, T., Ravanan, R., Venkatesan, P. Back calculation of HIV/AIDS in Tamil Nadu. In: Eds. Pandey, C. M., Mishra. P. and Singh, U. Biostatistical Aspects of Health and Epidemiology. Lucknow: Sanjay Gandhi Postgraduate Institute of Medical Sciences; 2002. p. 232-243.
2. Geetha Ramachandran, Prema Gurumurthy. Purification of cytochrome P-450 in mycobacteria. *Current Science, 2002,* **83**, 873-877**.**
3. Geetha Ramachandran, Prema Gurumurthy. Effect of rifampicin and isoniazid on Cytochrome P-450 in mycobacteria. *Indian Journal of Medical Research, 2002*, **116,** 140-144.
4. Paramasivan, C. N. The need for alternative to BCG. In : Drs. Raghunath & R Nayak, editors. Second Sir Dorabji Tata Symposium Series, 2001, New Delhi: Publishers : Tata McGraw – Hill Publishing Company Ltd.; 2002. p. 231-237.
5. Ramachandran, R., Paramasivan, C. N. What is new in the diagnosis of Tuberculosis? Part I : Techniques for diagnosis of tuberculosis: Part II: Techniques for drug susceptibility testing. *ICMR Bulletin 2002,* **32**,(8,9).
6. Ranjani Ramachandran, Soumya Swaminathan, Sulochana Somasundaram, Azgar, V. N., Paramesh, P., Paramasivan, C. N. Mycobacteremia in Tuberculosis patients with HIV infection. *Indian Journal of Tuberculosis, 2002,* **50**, 29-31.
7. Senthil Kumar, K. S., Uma Devi, K. R., Raja Alamelu. Isolation and evaluation of diagnostic value of two major secreted proteins of *Mycobacterium tuberculosis. Indian Journal of Chest Disease and Allied Sciences, 2002,* **44**, 225-232.
8. Shibi Chakravarthy, K., Raj B Singh, Soumya Swaminathan, Venkatesan, P. Prevalence of asthma in urban and rural children in Tamil Nadu. *The National Medical Journal of India, 2002,* **15**, 260-263.
9. Soumya Swaminathan, Sangeetha, M., Arunkumar, N., Menon, P. A., Beena Thomas, Shibi, K., Ponnuraja, C., Rajasekar, S. Pulmonary Tuberculosis in HIV individuals; Preliminary report on clinical features and response to treatment. *Indian Journal of Tuberculosis, 2002,* **49**, 189-193.
10. Uma Devi, K. R., Ramalingam, B., Alamelu Raja. Qualitative and quantitative analysis of antibody response in childhood tuberculosis against antigens of *Mycobacterium tuberculosis. Indian Journal of Medical Microbiology 2002,* **20**, 145-149.
11. Venkatesan, P. Methods for projection of HIV/AIDS epidemic. In : Anilkumar, editor. Epidemiology, Health and Population, Delhi: B R Publishing Group; 2002. p. 143-156.

12. Aziz, M. A., Ba, F., Becx-Bleumink, M., Bretzel, G., Humes, R., Iademarco, M. F., Kim, S. J., Lamothe, F., Paramasivan, C.N., Ridderhof, J., Sloutsky, A., van Deun, A., Shah, K.V., Weyer, K. External quality assessment for AFB smear microscopy. Association of Public Health Laboratories, NW; 2002.

13. Soumya Swaminathan. Basic concepts in the treatment of tuberculosis. *Indian Journal of Pediatrics, 2002,* **69**, 44-49.

14. Geetharamani Shanmugam. Awareness on HIV/AIDS among rural women in a south Indian community.
Indian Journal of Sexually Transmitted Diseases, 2002, **23,** 26-30.

15. Prema Gurumurthy, Hemanth Kumar, A. K., Annabelle Rajaseharan, Fathima Rehman, Sekar, L., Narayanan, P. R. Pharmacokinetics of ofloxacin, rifampicin, isoniazid and pyrazinamide when administered alone and in combination.
Biomedicine, 2002, **22**, 13-26.

16. Selvakumar, N. Role of laboratory in RNTCP. *Journal of Indian Medical Association, 2003,* **101,** 173-174.

17. Paramasivan, C. N. Status of drug resistance in tuberculosis after the introduction of rifampicin in India. *Journal of Indian Medical Association, 2003,* **101,** 154-156.

Accepted for publication

International

1. Marisa L. Pedulla, Michael E. Ford, Jennifer M. Houtz, Tharun Karthikeyan, Curtis Wadsworth, John A. Lewis, Debbie Jacobs-Sera, Jacob Falbo, Joseph Gross, Nicholas R Pannunzio, William Brucker, Vanaja Kumar, Jayasankar Kandasamy, Lauren Keenan, Svetsoslav Bardarov, Jordan Kriakov, Jeffrey G Lawrence, William R Jacobs Jr., Roger W Hendrix, Graham F Hatfull. Origins of highly mosaic Mycobacteriophage Genomes.
Cell.

2. Ramandeep Singh, Vivek Rao, Shakila, H., Radhika Gupta, Aparna Khera, Neeraj Dhar, Amit Singh, Anil Koul, Yogendra Singh, Naseema, M., Narayanan, P. R., Paramasivan, C. N., Ramanathan, V. D., Anil K. Tyagi. Disruption of mptpB impairs the ability of *Mycobacterium tuberculosis* to survive in guinea pigs.
Molecular Microbiology.

3. Daisy Vanitha, J., Venkatasubramani, R., Dharmalingam, K., Paramasivan, C. N. Large Restriction Fragment Polymorphism analysis of *Mycobacterium chelonae* and *Mycobacterium terrae* isolates.
Applied and Environmental Microbiology.

4. Selvakumar, N., Prabhakaran, E., Fathima Rahman, Naik Ashok Chand, Srinivasan, S., Santha, T., Chauhan, L. S., Thomas R Frieden, Narayanan, P. R. Blinded rechecking of sputum smears for acid fast bacilli to ensure quality and usefulness of re-staining of smears to assess false positive errors.
International Journal of Tuberculosis and Lung Disease.

5. Sudha, G., Nirupa, C., Rajasakthivel, M., Sivasusbramanian, S., Sundaram, V., Bhatt, S., Subramaniam, K., Thiruvalluvan, E., Mathew, R., Renu, G., Santha, T. Factors influencing the care-seeking behaviour of chest symptomatics : a community-based study involving rural and urban population in Tamil Nadu, South India.
Tropical Medicine and International Health.

6. Narayanan, P. R., Renu Garg, Santha, T., Paul Kumaran, P. Shifting the focus of tuberculosis research in India.
Tuberculosis.

National

1. Selvaraj, P., Chandra, G., Sunil Mathan Kurian, Reetha, A. M., Narayanan, P. R. Association of vitamin D receptor gene variants of *Bsm*I, *Apa*I and *Fok*I polymorphisms with susceptibility or resistance to pulmonary tuberculosis.
Current Science.

2. Soumya Swaminathan, Kuppu Rao, K. V., Somu, N., Vijayan, V. K. Reduction in work capacity in children with non cystic fibrosis bronchietasis.
Indian Journal of Paediatrics.

3. Tuberculosis Research Centre, Chennai. Trends in initial drug resistance over three decades in a rural community in south India**.**
Indian Journal of Tuberculosis.

4. Narayanan, P. R., Santha, T., Paul Kumaran P. Tuberculosis control strategies: Challenges to health management research.
Health Administrator.

5. Venkataraman, P. Paramasivan, C. N. Drug resistance in tuberculosis and issues related to multi-drug resistance for planning for TB control in India.
Health Administrator.

PARTICIPATION OF SCIENTISTS IN CONFERENCES / SEMINARS ETC.

International

1. Workshop on Cultural Epidemiology organized by the Swiss Tropical Institute and the UNDP/World Bank/WHO. Special programme for research and training in tropical diseases, Basel, Switzerland, 22-26 July, 2002 – M. S. Jawahar.
2. Summer School: Good Lung Function Practice, European Respiratory Society, Academic Medical Centre, Amsterdam, The Netherlands, 24th-30th August, 2002 – K. V. Kuppu Rao.
3. 33rd IUATLD World Conference on Lung Health Montreal, Canada 6-10 October, 2002 – C. N. Paramasivan, N. Selvakumar.
4. Role of stimulation studies in predicting the efficacy of anti-TB treatment regimens, Centres for Disease Control, Atlanta, Georgia, 16 October, 2002 – C. N. Paramasivan.
5. Fifth meeting of the Technical Review Committee for the Global TB Drug facility, Geneva, 30 October – 1 November, 2002 – T. Santha Devi.
6. WHO-UNAIDS workshop on Application of the IFN-gamma ELISPOT assay to monitor immune responses for HIV vaccine research in Asian countries, Beijing, China, 13-18 January, 2003 – Soumya Swaminathan.
7. Basic and Advanced training on the Beckman Coulter Epics Altra flowcytometer at the Beckman Coulter facility at Miami, Florida, USA, 13-24 January, 2003 – Sudha Subramanyam.
8. Scientific Writing Workshop on Gender-sensitive Interventions in Tuberculosis Control organised by the Swiss Tropical Institute and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, Basel, Switzerland, 20-24 January, 2003 – M.S. Jawahar, Sudha Ganapathy.
9. 10th International Conference on Retroviruses and Opportunistic Infections, Boston, USA, 10-14 February, 2003 – Soumya Swaminathan.

National

1. The Health InterNet Planning workshop, Bangalore, 2-3 May, 2002 – T. Santha Devi.
2. Seminar on Improving productivity in pharmaceutical development conducted by Waters (India) Pvt.Ltd., Chennai, 17 May, 2002 – Lalitha Victor, A. K. Hemanth Kumar, K. Silambuselvi.
3. First International Conference in Medical Sociology on Health, Illness and Society in the new millennium Madras Medical Mission, Chennai, 25-26 May, 2002 – Sudha Ganapathy and Geetharamani Shanmugam

4. Assessing the Sexual and Psychological Health of HIV positive Women in India – A multivariate analysis on HIV AIDS. UCLA/ICMR Workshop, Goa, 28-29 May, 2002 – P. Venkatesan.

5. National Seminar and workshop on Genetic Disorders and Diagnosis (DIAGNOGene 2020), Sri Ramachandra Medical College & Research Institute, Chennai, 29-31 May, 2002 – P. Venkatesan.

6. CME programme on RNTCP for the Independent Medical Practitioner's Association organized by REACH, Chennai, 16 June, 2002 – M. S. Jawahar.

7. National Seminar on Applications of Survival Analysis, NIMHANS, Bangalore, 21-22 June, 2002 – C. Ponnuraja, D. Vijaya Bhaskara Rao, Arun Kumar.

8. Workshop on Information and Communication held at National Institute of Occupational Health, Ahmedabad, 25-27 June, 2002 – Sudha Ganapathy.

9. Public hearing on Female foeticide and infanticide organized by the State Commission for Women at Chennai, 10 July, 2002 – Geetharamani Shanmugam

10. Workshop on Molecular Epidemiology of diarrhoeal diseases with special reference to cholera, at National Institute of Cholera and Enteric Diseases, Kolkata, 12-21 August, 2002 – N. S. Gomathi.

11. Workshop on ARV Therapy in Resource limited settings organized by The Tamilnadu Dr.MGR Medical University, Chennai, August, 2002 – Soumya Swaminathan, C. Padmapriyadarsini, Sheikh Ilyas, Beena E. Thomas.

12. XVII TNAI Biennial conference, Sri Ramachandra Medical College & Research Institute, Porur, 29 August, 2002 – Valarmathi Nagarajan.

13. Workshop on Patent Awareness for women scientists organized by the Dept. of Science and Technology, Meenakshi College for Women, Chennai, 5 September, 2002 – Geetharamani Shanmugam.

14. Seminar on Patent Information for Drug Research conducted by URDIP, Pune, 11-12 September, 2002 – Vanaja Kumar.

15. National meeting organized by Indian society of health administrators on TB management in India: Assessment, diagnostic and treatment Bangalore, 10-12 September, 2002 – Rajeswari Ramachandran, N. Selvakumar.

16. 57th National Conference on Tuberculosis and Chest Diseases, Panaji, Goa, 26–29 September, 2002 – Prema Gurumurthy, A.K. Hemanth Kumar, Ranjani Ramachandran, Sulochana Somasundaram, N. S. Gomathi.

17. Workshop on Social work education organized by Tamil Nadu State Non Governmental Organizations and Voluntary Resource Centre at the Tamil Nadu Corporation for the development of women at Chennai, 27 September, 2002 – Geetharamani Shanmugam.

18. Workshop on Effective Biomedical Communication at JIPMER, Pondicherry, 4 October, 2002 – A. K. Hemanth Kumar.

19. 23rd Annual Conference of the Indian Association of Biomedical Scientists (IABMS) at JIPMER, Pondicherry, 5-6 October, 2002 – A. K. Hemanth Kumar.

20. National Level Conference on Campaign for Custodial Justice and Abolition of Torture, Chennai, 11 October, 2002 – Jemima Sheila Fredrick.

21. National Pediatric Pulmonology Conference, Kottayam, 18-19 October, 2002 – Soumya Swaminathan.

22. National Workshop on Good Laboratory Practice (GLP), Chennai, 24-26 October, 2002, Chennai – M. Kannapiran, Lalitha Victor.

23. National Workshop for Nodal Centres for Medical Colleges in RNTCP, 29-31 October, 2002 – Rajeswari Ramachandran.

24. Comprehensive International Program of Research on AIDS (CIPRA) at National AIDS Research Institute (ICMR), Pune, 8 November, 2002 – Alamelu Raja.

25. RNTCP Research Dissemination Seminar organized by DANTB, Delhi, 21 November, 2002 – Rajeswari Ramachandran.

26. 26th IAMM conference held at NIMHANS, Bangalore, 22-24 November, 2002 – C.N. Paramasivan, Ranjani Ramachandran, G. Kubendran, Daisy Vanitha.

27. XXIX Annual meeting of the Indian Immunology Society & Symposium on Immunoparasitology at Regional Medical Research Centre (ICMR), Bhubaneswar, 27-29 November, 2002 – Alamelu Raja, V. D. Ramanathan, P. Selvaraj, K. Silambuselvi., C. Prabha, R. Priya.

28. Liquid Chromatography School conducted by Waters India Ltd., Chennai, 2-4 December, 2002 – Alamelu Raja, G. Kubendran, Daisy Vanitha.

29. National Seminar on Human Rights and Hunger organized by Professional Social Workers Forum at Madras School of Social Work, Chennai, 7 December, 2002 – Sudha Ganapathy, Geetharamani Shanmugam, Jemima Sheila Fredrick, Chandra Suresh, P. Murugesan.

30. Seminar on Adoption – creating a nurturing environment organized by Balamandir Research Foundation, Chennai, 14 December, 2002 – P. Murugesan.

31. Second Winter School in Immunology, organized by Harvard Medical School, USA, and International Centre for Genetic Engineering and Biotechnology, Goa, 15-21 December, 2002 – R. Priya, Kaustav Nayak.

32. Seminar on Counselling for Youth organized by the Madras School of Social Work and Rajiv Gandhi National Institute of youth development, Chennai, 19 December, 2002 – Geetharamani Shanmugam, P. Murugesan.

33. IRMS Silver Jubilee and XX Annual Conference of ISMS, New Delhi, 19-22 December, 2002 – P. Venkatesan, V. Chandrasekaran, C. Ponnuraja, L. Sekar, D. Vijaya Bhaskara Rao.
34. International Conference on Operations Research and Development, Anna University, Chennai, 27-30 December, 2002 – P. Venkatesan.
35. Epidemiology week organized by National Institute of Epidemiology, Chennai, 30 December, 2002 – 3 January, 2003 – M. S. Jawahar.
36. Annual Conference of the Indian Academy of Pediatrics, Mumbai, 2 January, 2003 – Soumya Swaminathan.
37. TRC-ICMR exhibition at the Science Congress, Bangalore, 3-7 January, 2003 – Prema Gurumurthy, V. D. Ramanathan, P. Selvaraj G. Kubendran, Nambirajan.
38. Second Indo-US Cytometry Workshop, CCMB, Hyderabad, 6-10 January, 2003 – Alamelu Raja.
39. International Conference on Chest Diseases and Allied Sciences, New Delhi, 12-14 January, 2003 – P. Selvaraj.
40. UGC workshop on Advanced Computer methods, Madurai Kamaraj University, Madurai, 13-14 February, 2003 – P. Venkatesan.
41. Indo-Norwegian seminar on Recent Trends in Tuberculosis Research, IIT, Kharagpur, 15-16 January, 2003 – S. Selvakumar, C. Prabha.
42. Training Programme on Certified Quality Manager, Jaipur, 10th – 22 February, 2003, Jaipur – M. Kannapiran.
43. National Symposium on Molecular Biology-50 years progress organized by the Ethiraj College for Women, Chennai, 20-21 February, 2003 – Kaustuv Nayak, N. M. Subramanian, Jenifer Jancy Rani.
44. A two-day Symposium on Proteomics and Hands-on Training Course on Proteomics, CCMB, Hyderabad, 23 February – 10 March, 2003 – Alamelu Raja.
45. Seminars on Research methodology, Sri Ramachandra Medical College & Research Institute, Chennai, 17-18 March, 2003 – P. Venkatesan.
46. Lepra India meeting held on World TB Day in Hyderabad, 22 March, 2003 – C. N. Paramasivan.
47. Workshop on TB challenges and NGOs involvement organized by the Social work of ICMR/TRC Madurai Unit, 29 March, 2003 – Geetharamani Shanmugam

Conferences/workshop/seminars, etc. organized:

1. Scientific writing workshop conducted by CDC/ WHO/TRC/ICMR, Chennai, 1-5 April, 2002.
2. National Symposium on Recent Advances in Statistical Computing, TRC, Chennai, 19-20 June, 2002.
3. TB challenges & Role of NGOs by TRC at Madurai on 1st December, 2002.

STAFF DEVELOPMENT PROGRAMMES

Staff and students who have been awarded Ph.D. degree by The Tamil Nadu Dr.M.G.R.Medical University, Chennai during the year:

1. Dr. A. K. Hemanth Kumar - April 2002 – "Biochemical and pharmacological aspects of ofloxacin in tuberculosis".
2. Dr. Luke Elizabeth Hanna - May 2002 – "Compartmentalisation of immune responses in lymphatic filariasis and tuberculous lymphadenitis".
3. Dr. Sunil Mathan Kurian - October 2002 – "Studies on human leucocyte antigen (HLA) and non-HLA gene polymorphisms on lymphocyte response to *Mycobacterium tuberculosis* antigens in spinal tuberculosis".
4. Dr. Cheruvu Mani – February 2003 – "Rapid identification of multi-drug-resistant *Mycobacterium tuberculosis* - development of a new tool and comparison with other methods".

Staff who have been awarded post graduate – degree

1. Dr. Paul Kumaran awarded MPH Degree. "Assessment of community infection ratio in the "Model Dots Project" area for tuberculosis control" by SCTIMST, Trivandrum
2. N. S. Gomathy – M.Sc.- Applied Microbiology – Madras University
3. Gomathy Sekar – M.Sc. – Applied Microbilogy – Madras University
4. Mariam George – M.Sc. – Medical Technology – PGI, Chandigar
5. Nalini Sunder Mohan – M.Sc. – Zoology – Annamalai University

Staff who have completed 1 yr certificate course

1. K. Ramakrishnan, C.M.L.T.
2. D. Venugopal, C.M.L.T.

Students who have submitted Ph.D. Thesis

1. Mr. S. Selvakumar, March 2002- The Tamil Nadu Dr.M.G.R. Medical University, Chennai.
2. Mr. V. Kamalakannan, April 2002 - University of Madras, Chennai.
3. Ms. J. Daisy Vanitha, July 2002 - The Tamil Nadu Dr. M. G. R. Medical University, Chennai.
4. Mr. B. Ramalingam, December 2002 – The Tamil Nadu Dr. M. G. R. Medical University, Chennai.

Students doing Ph.D. degree:

G. Radha, M.Sc.	CSIR- Senior Research Fellow
V. Aravindhan, M.Sc.	CSIR- Senior Research Fellow
Deepak Jayakumar, M.Sc.	CSIR- Junior Research Fellow
Dr. P. L. Natarajan, M.B.B.S.	CSIR- Junior Research Fellow
D. Anbarasu, M.Sc.	CSIR- Junior Research Fellow
R. Priya, M.Sc.	ICMR- Junior Research Fellow
C. Prabha, M.Sc.	ICMR- Junior Research Fellow
Nisha Rajeswari, M.Sc.	ICMR- Junior Research Fellow
M. Vidya Rani, M.Sc.	ICMR- Junior Research Fellow
G. Chandra	UGC- Senior Research Fellow

Staff doing Ph.D. degree

M. Muniyandi, M.A., M.Phil., M.P.S.

Sulochana Somasundaram, M.S., C.M.L.T.

L. Prabakaran, M.Sc.

R. Selvaraj, M.Sc.

Beena E. Thomas, M.A.

C. Ponnuraja, M.Sc.

N. S. Gomathy, M.Sc.

Gomathi Sekar, M.Sc.

K.Chandrasekaran, M.Sc.

N. Arunkumar, M.Sc.

J. Daisy vanitha, M.Sc.

Awards/Honours received:

1. Second Best Poster award for the paper entitled, "Vitamin D receptor gene polymorphisms (*Bsm*I, *Apa*I and *Fok*I) in pulmonary tuberculosis" by **Selvaraj**, **P.,** Chandra, G., Sunil Mathan Kurian, Reetha, A.M., Narayanan P.R. at the XXIX Annual meeting of the Indian Immunology Society & Symposium on Immunoparasitology, Regional Medical Research Centre (ICMR), Bhubaneswar, 27-29 November 2002.
2. Second Best Poster award for the paper entitled "Rapid identification of multi-drug resistant tuberculosis" by **Cheruvu Mani**, Selvakumar, N., Vanaja Kumar, Sujatha Narayanan, Narayanan, P.R. at the National level conference on "50 years of DNA" held at Ethiraj College for Women, Chennai, Feb. 2003.

STAFF LIST

Director

P. R. Narayanan, Ph.D.

Deputy Director (Senior Grade)

C. N. Paramasivan, Ph.D., D.Sc., F.A.M.S.
T. Santha Devi, M.B.B.S., D.T.C.D.
Aleyamma Thomas, M.D., Dip.in.Lep.
V. Kumaraswami, M.D., M.N.A.M.S., Ph.D.(Med.)
Rajeswari Ramachandran, M.D., D.M.(Neuro.), Ph.D.
Rani Balasubramanian, M.D., D.G.O.
M. S. Jawahar, M.D., M.Sc., D.L.S.H.T.M.

Deputy Director

V. D. Ramanathan, M.B.B.S., Ph.D.
Prema Gurumurthy, Ph.D.
Soumya Swaminathan,M.D., Dip.N.B.
N. Selvakumar, Ph.D.
Vanaja Kumar, Ph.D.
Rema Mathew M.B.B.S.,D.C.H.
Alamelu Raja, Ph.D.
K. Rajaram, B.Sc.,M.B.B.S., D.T.R.D.
Pauline Joseph, M.B.B.S.,D.D.
C. Kolappan, M.B.B.S., M.Sc.(Epid)
K. Sadacharam, M.B.B.S., D.P.H.
Sujatha Narayanan, Ph.D., C.T.(ASCP)

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R. Subramani, M.Sc.
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)

Senior Research Officer

R. Selvaraj, M.Sc.
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Ranjani Ramachandran, M.D.
K. V. Kuppu Rao, Ph.D.
Lalitha Hari, M.Sc.
D. Sulochana, Ph.D.
Chandra Immanuel, M.Sc.
P. Paul Kumaran, M.B.B.S., M.P.H.
Pradeep Aravind Menon, M.B.B.S.
D. Baskaran, M.B.B.S.
C. Padma Priyadarshini, M.B.B.S., D.N.B.

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Geetha Ramachandran, Ph.D.
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Senior Technical Officer

Sudha Ganapathy, M.A.
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R. S. Nagabhushana Rao, B.Com.
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K. Jayasankar, Ph.D.
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Lalitha Victor, M.Sc.
K. J. Ilampuranam, B.Sc.
S. Ramanujam, B.Sc., D.M.L.T.
Victor Mohan, B.Sc.

Nursing Officer

Jayalakshmi Vadivel, B.Sc.

Technical Officer

E. Money
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H. Dhanasekaran
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R. Sashidharan, B.Sc.
B. S. Phaniraj, B.A.
V. Venkatesh Prasad, B.Sc.
R. Chandra Mohan, B.Sc.
R. Krishnamoorthy, B.A.
P. Narayanan
G. K. Loganathan, M.A.
G. Baskaran, M.Sc.
Vasanthira Patturaj
Santhamma Asokan
S. C. Ramaiah
K. Padmavathy
S. Sathiamoorthy, B.A.
N. Ganesan, B.A.
L. Krishnamacharya, B.Sc.
B. Radhakrishna, B.A.
S. Radhakrishnan, B.A
V. Sundaram, B. Sc.
K. Gopala Jetty
K. Jacob, B.Sc.
K. Sankaran, B.Sc., M.L.T
V. Chandrasekaran, Ph.D.
M. Ponnambalam, B.Sc
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E. Munusamy, M.A.

Senior Public Health Nurse

B. V. S. Chalapathy Rao, B.Sc.
Arumaikannu Anbalagan, B.Sc.

Research Assistant

R. Segaran, M.Sc.
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K. Subramanian, M.Sc., M.Ed.
M. Subhas Chandra Bose, M.Sc.
D. Suryanaraynan, M.Sc.
L. Sekar, M.Sc.
Sripad Bhat, M.Sc.
D. Vijayabhaskara Rao, Ph.D.
K. Chandrasekaran,M.Sc.
G. Komaleeswaran, M.Sc.
G. Kubendran, Ph.D.
Naik Ashok Chandu, M.Sc.
K. Silambuselvi, M.Sc., M. Phil.
N. Arunkumar, M.Sc.
A. K. Hemanth Kumar, Ph.D.
Sulochana Somasundaram, M.S., C.M.L.T.
S. Selvakumar, M.Sc.
J. Daisy Vanitha, M.Sc.
M. Muniyandi, M.A., M.Phil., M.P.S.

Medical Social Worker

Beena E. Thomas, M.A.
Mohana Rani Suhadev, M.A.
K. J. Jaganatha Rao, M.A
Rajasakthivel, M.A.
E. Thiruvalluvan, M.A.
Meenalochani Dilip, M.A.
Chandra Suresh, M.A.
D. Kalaiselvi, M.A.
P. Murugesan, M.A.

Public Health Nurse

A. Gunasundari, B.Sc.
G. Mangalambal, B.Sc.
N. Valarmathi, B.Sc.
C. Kavitha, B.Sc.
Susan Stella Bai

Console Operator

S. Boopathy, B.Sc.

Clinic Nurse

K. K. Valsamma
Emily Verghese
V. Meenakshi
P. Muthulakshmi
S. Padma
Padma Prakash
Mary Eunice George
S. Chellam
R. Mirunalini
Victoria Kamalam Jeyaraj
K. Rosily
Nagalakshmi Janaradhana Reddy
A. Komathi, B.Sc.

Technical Assistant

D. Vijayakumar
S. Janakiraman, B.A.
R. Ravichandran, B.Sc.
V. Ramanathan
V. Sabapathi
CH.P.Prakashkumar, M.A.
M. Mubarak Ali
K. Singaravelu, M.A.

V. Vasudevan, M.A.
P. Venkataraman
K. Somasekar
V. Kusalakumaran, B.A.
S. Kumar, B.Com
Mohammed Amanullakhan
C. Packirisamy
T. Vamanamurthy
A. Vedachalam
S. Stanley Jones Rajasingh, B.Sc.
B. Senthilkumar, B.A.
K. Kathirvel, B.A.
M. Kalyanaraghavan, M.Sc.
S. Egambaram, M.A.
A. S. Tholkappian, B.Com.
P. Nagarathinam, B.A.
D. Benjamin, M.A.
M. Gopalakannan, B.A.
A. S. Kripasankar, B.Sc.
Jemima Sheila Fredrick
S. I. Eusuff, M.A., B.L., PG Dip. PM & IR.
V. Sampathkumar, B.A.
P. G. Shanmugam, B.Sc., B.L.
D. Sarkunan, B.Sc.
K. Balasubramaniam, B.Sc.
G. Prabhakar, B.A.
E. Sathiyavelu, M.A.
K. R. Ravichandran, B.Sc.
Malathy Parthasarathy, B.Sc.
K. Sampoornam
G. Hemavathy
Catherine Bosco
Anna Anthony
D. Pachayappan
A. Gomathy
Shyamala Gopu, M.A.
R. K. Rajendran
E. Kirupakaran
B. Kanagasabapathy, M.A.
R. Manoharan
A. Syam Sundar, D.M.L.T.
S. Jagadeesan
P. Jagannathan
C. V. Mohan, B.A.
S. Ravindra Rao, M.A.
N. Saradha, B.Sc., D.M.L.T.
J. Samuel Vasanthan Goodwill, B.Sc.Ed., D.M.L.T.
N. S.Gomathy, M.Sc., D.M.L.T.
Gomathi Sekar, M.Sc., C.M.L.T.
K. Govindharajulu
V. Partheeban, M.A.
S. Manoharan, B.Sc., D.M.L.T.
K. Rajagopal, B.Sc., D.M.L.T.
Rajeswari Balasubramanian, D.M.L.T.
Beulah Jasmine
J. David Silver Durai
E. Prabhakaran
S. Mohd Ghouse
T. Gowrisankar
V. Revathy
R. Valarmathi

Data Entry Operator

S. Gopalakrishnan
V. Subramanian
T. Raman
M. John Jayaraj
S. Vijaya
G. Thangam, B.Sc.

Laboratory Technician

Lakshmi Sambandam
Sivagama Sundari, C.M.L.T.
L. Prabakaran, M.Sc., D.M.L.T.
Mariam George, M.Sc., C.M.L.T.
M. Anandan, C.M.L.T.
K. Ramakrishnan, M.Sc., D.M.L.T.
M. Subramani
C. ThiruKumar, B.A., C.M.L.T.
K. Devika, B.Sc., C.M.L.T.
Mohammed Shahabuddin
V. N. Azgar Dusthakeer, B.Sc., C.M.L.T.
D. Thangaraj, C.M.L.T.
V. Girijalakshmi, B.Sc., C.M.L.T.
D. Saraswathi, M.Sc., C.M.L.T.
S. Nambirajan B.Sc., D.M.L.T.
K. Balakaliyan, B.Sc.
T. Narayanan
M. Parthasarathy
K. Rajasekaran, C.M.L.T.
M. Ashokan
N. Rajendran, C.M.L.T.
B. Daniel, C.M.L.T.
M. Baskaran, B.Sc., C.M.L.T.
S. Rajakumar, B.Sc., C.M.L.T.
B. Angairkanni, M.Sc.

V. Thyagarajan, B.Sc., D.M.L.T.
J. Chitra, B.Sc., D.M.L.T.
N. Lakshmikanthan
M. Jayapalan
P. Jayaram, B.Sc.
C. Suganthi, B.Sc.
P. L. Vasuki, B.Sc.
M. Malathi, B.Sc., C.M.L.T.
D. Ravikumar, B.Sc., C.M.L.T.
Murugesan, M.Sc., C.M.L.T.

Technician

V. Revathy
R. Valarmathy
V. Indirani
K. Palaniyandi
R. Padma
R. Saraladevi
V. Farthimunnisa
P. Pandeeswari
M. Rathinam
S. Theensuwai
P. Kowsalya
M. Mohana
R. Kuthosh
V. Ramesh babu
P. Munivaradhan, B.Sc.
K. Ranganathan, B.A.
D. Nithyakumar, M.Sc.
R. Vetrichelvi, M.A.
R. Manimegalai
Varadharajan Shakila
S. V. Joseph Rajkumar
P. K. Venkataramana , B.Com.
N. Prem Kumar,B.Sc.
S. Venkatesan , B.Sc., B.Ed., M.A.
A. M. Ramesh, M.A.
T. Thangaraj, B.Sc., B.Ed.

Dark Room Assistant

N. Shanmugam
E. A. John Washington

Laboratory Assistant

N. Jayaraman
M. Mohan
A. Durairaj
C. Sivan
K. Krishnan
K. Ramakrishnan
N. Thangavelu
M. Michel Prem Kumar, B.Sc., C.M.L.T.
P. Venkatesan

Senior Laboratory Attendant

G. Nithyanandam
C. Gopal
S. Sundararajan
M. Thanigachalam
D. Balakrishnan
B. Venkata Ramana Rao
M. K. Sugumar
C. C.B. Sathya Narayanan

Laboratory Attendant

S. Kumaran
K. Shanthi
V. Pandurangan
M. R. Kamalanaban
D. Venugopal
G. Kabirdass
G. Chandramouli
Srikant Davani
K. Munusamy
S. Meera Sahib
K. Chandran
S. Mookkan
E. Masilamani
Ishwori Dhakal
R. Krishna Bahadur
R. Gangador Sharma
A. Pratap singh
N. Ramakrishnan
A. Manoharan
P. Chandran
J. Udhaya kumar
V. Raja

Nursing Orderly

Padmavathi Asaithambi
G. Sakunthala
Rosily Edwin
K. Jayavel Anandan
D. Sundari

Senior Administrative Officer

M. Subramanian, B.Com.

Administrative Officer

V. Adikesavan, B.A.

Accounts Officer

N. C. Sridharan, B.Com.

Purchase Officer

Padma Balasubramanian

Section Officer

P. K. Srinivasan, B.Sc.
B. Ramdoss, B.A.
M.R.Srinivasan
K. Jayarajan
D. Ramani Bai
S. Vasantha
M. Mani, B.A.
R. Ramamirtham
A. Abdul Rahman, B.Sc.

Private Secretary

M. Vijayalakshmi
Ranajith Sankar Sen
T. M. Kasinathan, B.Com., PG Dip. PM & IR
Jothi Segaran

Assistant

K. Sampath Kumar, B.Sc.
A. V. Samuel Swamidoss, B.A.
Santhivelu, M.A.
D. Arul Doss, B.A.
Y. Sam Wilson, B.A.
K. Karunakaran, B.A.
P. N. Kalavathi Chari
K. Kuppuswamy, B.A.
G. Sundaresan, B.Sc.
K. Nagamony, B.A.
V. Lalithamma
D. Devaki, B.A.
B. Nazeema Beevi, B.Sc.
M. J. Nirmala, B.A.
M. Meenal, B.Com.
R. Visalakshi, M.A.

Personal Assistant

S. Rangamma
Santha Sriraghavan
K. Saroja
P. Karthigeyan, B.Com.
B. Doraiswamy, B.A.
V. R. Vijayalakshmi

Senior Receptionist & T.O.

K. S. Anusuya

Senior T.O & Receptionist

Kanchana Udayakumar

Upper Division Clerk

T. N. Surendranath, B.Sc.
V. Elumalai
N. Tamil Selvi, B.Sc.
Beela Mohan, B.A.
Susheela Soundarajan, B.Sc.
D. Vijayakumari, B.A.
R. Lakshmi, M.A.
V. Selvaraju, B.A.
M. Rasheetha Begum, M.A.
C. Gopala Krishnan, B.Sc.
R. Geetha, B.Com.
Chithra Sivakumar, B.Sc.
S. Rajendran

Stenographer

Usha Devi Gopalan
Santhi Viswanathan
Stanley Gnanadhass, B.Sc.
A. L.Rajalakshmi, B.Sc., PGDCA

Telephone Operator

V. Shailaja Devi

Lower division Clerk

A. Lakshmanan
R. S Dayalakumar
A. K. Vijayal
L. Vijayakumari
M. J Nagalakshmi, M.A.
B. Durai Raj
P. Kowsalya B.A., Dip.CDCW
P. Kavitha, M.A.
B. Gopinath B.Com.
M. N. Raadha, B.Sc.

R. Senthil Nathan
S. Anandaraj
S. Nirmala, B.A.
A. S. Sivaraj, M.A.
J. Suguna, B.Com.
T. Sheela
K. Kanaga
A. Uma, B.Com.
M. Senthilkumar, B.Sc.

Gestetner Operator

B. Anbudoss
V. Damodharan

Senior Record Sorter

P. Velan
V. Adiseshan
S.M. Syed Noorudeen

Record Sorter

G. Subramanian
T. Thiru Gnanasambandam
Molly Joseph
K. Ganesan
J. Loganathan
G. Moshe
C. Nagaraju

Daftry

M. Ranibai
M. S. Devakumar
P. Vijayakumar
K. Meenakshi
A. Annamalai
V. Mohan
R. Damodharan
V. Velmurugan
R. Anbulingam
V. Navalan

Attender

Balakrishna Sharma
M. Seeli
Yam Bahadur
M. B. Mohanan
G. Gajavalli
R. Ravichandran
P. Chinniah
K. V. Rajamma
Jagat Bahadur
M. Kannan, C.M.L.T.
Soloman Priyakumar
Srinivasa Raju
V. Adikesavan
V. Sundarajan
K. Sumathy
R. Ganapathy
K. Kuttappan
P. Johnson Kennedy
E. Duraivel
J. Venkatesan
D. Bose
N. Murali
C. K.Chittarasu
M. Jayaraj
A. Rajavarman
J. Selvam
G. Easwaran
G.Nithyanandam

Transport Supervisor

V. K. Venkatesan
T. S. Mahadevan

Workshop Mechanic

R. Jayaganga Babu
V. Pakkiriswamy

Driver-cum-Mechanic

K. Parthiban
S. Iyyappan

Driver

A. Ganapathy
E. Raman
D. Chandran
G. Vasu
N. Govindarajulu
S. Ayyappan
S. Rajkumar
P. Philipose
M. Govindan
J. Krishnamurthy
S. Krishnan
K. G.Kanagasabapathy
K. Karunakaran
R. Nandan
R. Arthur Sundar Singh
K. Vadivel
K. Ayyasamy
C. Krishnamurthy

P. Anbu
S. Sri Ramachandran
P. Soundararajan
V. Thanigaivel
K. Venugopal
K. Jayaraman
J. Prakash
B. Kalaiselvam
B. Vijayakumar
A. Ravi
M. Manogaran
K. Saravanan
A. S.Dayalan
R. Balu
K. S.Venkatesan
B. Suresh Kumar
P. Subbaiah
K. Thulasingam
A. Elangovan
I. Seenivasan

Care Taker

D. Balraj
D. Shanmugam

Plumber

R. S. Soma Sundaram
J. Ravi

Electrician

K. Poongavanam

Cook

K. Kunhiraman
P. Madhan kumar

Sr.Helper

P. Ellamanda

Scout

P.C.Nagaraja

N. Srinivasan
K. Vasudevan
R. Purushothaman
J. Jeeva
S. Prakasam
F. Albert
G. Vasu

Watchman

Min Bahadur
Jeevanath Sharma
Keshabraj Paudel
V. Pakkiriswamy
C. Uthara Bahadur

Chowkidar

Hariprasad Sharma
M. John Robert
Til Bahadur
T. D.Ponnuswamy
R. Yuvarajan

Sweeper

K. V. Nageshwar Rao
D. Achamma
G. Thirupal
Penchillamma
R. Ankamma
K. Ragammal
R. Ankaiah
A. Pandy
D. Sharadha
N. Ankaiah
B. Nageswari
C. Anandan
Kasiammal
P. Arul Mani
G. Devaki
S. Lakshmi
N. Vasantha
G. Durai
V. Venkateswaralu
J. Neelavathy
H. Ponrose
T. Thilakavathy