Annual Report 2012-13

NIRT

National Institute for Research in Tuberculosis

WHO Collaborating Centre for Tuberculosis Research & Training
International Centre of Excellence in Research

NATIONAL INSTITUTE
FOR RESEARCH IN TUBERCULOSIS

Research Activities
April 2012 March 2013

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PREFACE

Over the past year, the institute has consolidated its research efforts towards developing shorter, more effective regimens for pulmonary tuberculosis (PTB) in HIV uninfected adults and towards defining the optimal treatment regimens for HIV-associated TB. The Clinical Research division also launched a new randomized clinical trial to shorten chemotherapy in TB lymphadenitis, enrolling patients with confirmed TB lymphadenitis. The institute thus continues its quest for better treatment options for all forms of TB and will pursue the investigation of new drugs as they become available. Research is also ongoing in defining predictive markers for immune reconstitution syndrome, a debilitating condition commonly occurring in HIV-infected patients with advanced immunodeficiency, initiating TB and ant-HIV treatment together. Further, an NIH-funded multicentric study to identify host genetic and nutritional risk factors for metabolic syndrome and lipodystrophy in children with HIV infection, has been enrolling at 5 sites across south India. The study will look at polymorphisms in lipid metabolizing genes in HIV+ children, for the first time in India. A recently completed study also found that both iron deficiency anemia and anemia of chronic inflammation are common among these children and that iron supplementation along with antiretroviral treatment was effective in overcoming the anemia.

The clinical division has expanded its collaborative work and now has agreements with three medical colleges/research institutes, apart from the ongoing collaboration with the Tamil Nadu state government medical colleges, TNSACS and Corporation of Chennai. Patients attending these centres are screened and enrolled into clinical studies, and faculty from these centres is included as co-investigators. NIRT was identified by NACO as a centre of excellence for research and will use secondary program data for analysis.

Socio-behavioural research studies have focused on health seeking behavior and practices of vulnerable populations both for HIV and TB. Different strategies to reduce sexual risk taking behavior among men who have sex with men, including the use of mobile technology are being explored. Further, a randomized clinical trial is testing cognitive behavioural therapy to reduce alcohol dependence and thus improve TB treatment outcomes. A study of migrant brick kiln workers was completed and showed that the prevalence of chest symptoms was high and awareness of TB was low in this group. Community involvement in TB control is being explored via the use of self-help groups to improve case finding as well as directly observed treatment.

Clinical pharmacologic studies have been done to study adequacy of first line anti-TB drug levels in HIV infected and uninfected children with TB and the results have been communicated to the TB program managers. Further, a pilot study was undertaken in collaboration with GHTM, Tambaram to study Rifabutin (RBT) levels in patients on 2nd line ART. This is likely to lead to a larger, multicentric study across NACO sites in different states, to define optimal RBT dosages in Indian patients. Pharmacogenetics is also expanding and we have developed molecular assays to test for genetic polymorphisms in drug metabolizing enzymes for many anti-TB and antiretroviral drugs. These studies throw light on the need for higher/lower dosages than those recommended for Caucasian

populations and will have practical implications for management of patients in India.

The HIV laboratory performs three types of functions providing all the basic support laboratory investigations for NIRT study patients, serving as a referral laboratory for NACO for the southern region (DNA and RNA PCR) as well as engaging in basic research. The HIV lab is a Regional Centre of Excellence for NACO and provides results for the early infant diagnosis program and also viral load results for patients failing first line anti-retroviral treatment (ART), thus supporting the AIDS Control program in the state. Apart from drug resistance surveillance in the HIV population, the lab is engaged in research on subtype C strain and also HIV-2. Unique HIV-1 subtype C recombinants have been recently identified. Molecular characterization of HIV-1 subtype C isolates showed that the majority used CCR5 as co-receptor and has rev genes of variable length. Ongoing work will determine the functional significance of the C-terminus heterogeneity of HIV-1 subtype C rev, by cloning the rev proteins into a vector and co-transfection experiments.

The Department of Bacteriology has been busy performing the duties of a Supranational and National Reference laboratory for mycobacteriology. Reference panels are developed and shipped to Intermediate Reference laboratories (IRL), where on-site evaluations and external quality assurance are provided by NIRT. Further characterization of the new molecule “transitmycin” has been done and the substance will now be produced in bulk at > 97% purity in order to undertake further toxicity and efficacy experiments. Work is ongoing to improve the sensitivity of the luciferase reporter phage (LRP) assay both for rapid drug susceptibility testing (DST) from clinical specimens as well as a rapid diagnostic test. The department also took on the added responsibility of performing line probe assays for patients with suspected MDR-TB, to support the TB program in Tamil Nadu. Second line DST is performed on MDR-TB specimens received from 9 states.

Biostatistics has always been an important pillar, lending support to the multifarious research activities of the institute, both clinical and basic science. Apart from these supporting activities and involvement in data management, the department also has multiple research interests, especially in the areas of modeling, neural networks, survival analysis and geospatial mapping.

Research in the Immunology department covers different aspects of the immunology and molecular biology of TB. Focus areas include antigen detection as a diagnostic test for TB, the role of host genetics (cytokine polymorphisms, Vitamin D receptor polymorphisms) in susceptibility to TB among HIV+ and HIV- individuals, cell mediated immune responses in pleural TB and identification of virulence genes in M.tuberculosis. In collaboration with JALMA, newer vaccine candidates are being tested in guinea pigs and mice. The International Centre for Excellence in Research (ICER) has focused on the immunological changes occurring when parasitic infections like filariasis occur along with latent or active TB. The polarization of the immune response towards a Th2 phenotype leads to increased susceptibility to TB in people with latent TB infection. Further, a study of the impact of type 2 diabetes mellitus on the immune response to TB has shown that the inflammatory response is heightened, leading to potentially more severe forms of TB.

Prevalence surveys for TB, which were earlier conducted in Tiruvallur district, have been completed in Chennai city. The prevalence of TB was similar to that seen in earlier surveys (~330/100,000) and was higher in slum compared to non-slum areas and in men compared to women.

The institute conducted several workshops during the year, including a dialogue with private practitioners on how best to manage TB in the private sector topics discussed included diabetes and TB, childhood TB and extrapulmonary TB as well as the role of newer diagnostic tools. An Indo-US Science and Technology funded workshop examined the link between nutrition, TB and HIV and came up with four high priority research questions. Around World TB day 2013, a CME program on drug resistant TB was conducted in collaboration with the Institute of Thoracic Medicine and Madras Medical College. Our scientists served as faculty at many conferences and workshops around the country.

Research priorities for the future include testing new drugs and adjunctive therapies for TB, shortening treatment for TB in children, testing of new diagnostics, pharmacokinetic and pharmacogenetic studies, testing of new compounds against TB and HIV, studying risk factors for recurrence and behavioural interventions to reduce HIV risk behavior as well as improve TB treatment outcomes.

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DISTINGUISHED VISITORS
GUEST LECTURES

S.No.	Date	Name	Topic
1.	11.04.2012	Dr. Jerry Pierson, R.Ph., Ph.D. Chief Regulatory Compliance and Human Subjects Protection, NIAID Division of Clinical Research, Bethesda.	Lessons learnt in GCP
2.	23.04.2012	Dr. Prathap Tharyan MD Christian Medical College, Vellore	Directly observed therapy for treating TB
3.	10.09.2012	Dr. Lalitha Ramakrishnan Professor of Medicine, University of Washington, Seattle	New insights into tolerance in *Mycobacteria*
4.	05.10.2012	Dr Ram Prasad, Spinco Biotek	Next generation sequencing (Illumina – My seq)
5.	29.10.2012	Dr Charu Sharma Senior Scientist, CSIR IMTEK, Chandigarh	Interacting partners of Erp protein of *M.tuberculosis* protein with macrophage proteins
6.	09.11.2012	Dr. Amit Misra Pharmaceuticals Division, CDRI, CSIR, Lucknow	Evolving a plan of clinical studies on inhalable microparticles containing anti-TB Agents
7.	08.02.2013	Prof. Dr. Susanne Hartmann Freie Universität Berlin, Institute of Immunology Berlin, Germany	Immunomodulation : Lessons from parasitic nematodes
8.	25.02.2013	Prof. Shabaana Khader Assistant Professor of Pediatrics Department of Immunology University of Pittsburgh, Pennsylvania, USA	T-helper cells in immunity to TB

ABBREVIATIONS

Anti-retroviral treatment (ART)
Acid fast bacilli (AFB)
Acquired rifampicin resistance (ARR)
Alcohol dependence scale (ADS)
Alcohol use dependence (AUD)
Anti-TB treatment (ATT)

Bovine serum albumin (BSA)

Cationic anti-microbial peptide (CAMP)
Chronic pathology (CP)
Complementary DNA (cDNA)
Community Advisory Board (CAB)
Contact specific (CS)
Culture filtrate antigen (CFA)
Culture filtrate proteins (CFP)

Data and safety monitoring board (DSMB)
Designated microscopy centres (DMC)
Directly observed treatment (DOT)
Drug resistance surveillance (DRS)
Drug susceptibility testing (DST)

Efavirenz (EFV)
Ethambutol (EMB)
Extensive drug resistance (XDR)

Far western blotting (FWB)
Fetal calf serum (FCS)
Fine needle aspiration cytology (FNAC)
Fixed group discussions (FGDs)

Healthy controls (HCs)
Healthy household contacts (HHC)
High performance liquid chromatography (HPLC)

IFNγ release assays (IGRA)
Immune reconstitution inflammatory syndrome (IRIS)
Intermediate reference laboratory (IRL)
Isopropyl β-D-1-thiogalactopyranoside (IPTG)
Isoniazid (INH)
Isoniazid TB preventive therapy (IPT)

Latent TB (LTB)
Least squares support vector machine (LS SVM)
Line probe assay (LPA)
Lipoarabinimannan (LAM)
Liquid chromatography tandem mass spectrometry (LC/MS)
Lowenstein Jensen (LJ)
LJ with sodium pyruvate (LJ-SP)
Luciferase reporter phage assay (LRP)

Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF)
Men having sex with men (MSM)
Minimal inhibitory concentration (MIC)
Mothers living with HIV (MLH)
Moxifloxacin (MFX)
Multi-drug resistance (MDR)
Multiplicity of infection (MOI)

National Institute of Health (NIH)
Nevirapine (NVP)
New sputum smear positive cases (NSP)
Non-TB (NTB)

Ofloxacin (OFX)

People living with HIV (PLHIV)
Phenol ammonium sulphate (PhAS)
Phorbol myristate acetate (PMA)
Pleural fluid (PF)
Polymerase chain reaction based restriction fragment length polymorphism (PCR-RFLP)
Population proportion to size (PPS)
Pulmonary tuberculosis (PTB)
Pyrazinamide (PZA)

Randomized controlled trial (RCT)
Relative light units (RLU)
Re-treatment (RT)
Reverse transcription polymerase chain reaction (RT-PCR)
Revised National TB control program (RNTCP)
Rifabutin (RBT)
Rifampicin (RMP)
Ritonavir (RTV)

Serine / threonine protein kinases (STPK)
Selective Kirchner medium (SKM)
Self help groups (SHGs)
Sequential minimal optimization (SMO)
Signal recognition particles (SRP)
Support vector machine (SVM)
Strcutural risk minimization (SRM)

Toll like receptor (TLR)
Triglycerides (TGL)
T-regulatory (Treg)
Tuberculin skin test (TST)
Tuberculous pleurisy (TP)

World Health Organization (WHO)

Vitamin D receptor (VDR)

Ziehl-Neelsen (ZN)

1
CLINICAL STUDIES
Department of Clinical Research

OVERVIEW : The Department of Clinical Research is the fulcrum around which the National Institute for Research in Tuberculosis revolves. This department specializes in the conduct of randomized controlled clinical trials to address various priority issues in TB treatment. The strength of NIRT's clinic is its infrastructure consisting of physicians, nursing staff, social workers and field investigators. Clinical studies also focus on special situations like HIV/TB, Immune reconstitution, Pediatric TB, Pediatric HIV, Drug resistant TB etc. Most of the studies in the clinic address areas that would help improve the national programmes. The department collaborates with a number of state government institution and hospitals including Government General hospital, Chennai, Kilapuk Medical college & hospital, Chennai, Govt. Stanley Medical college and hospital, Govt. Hospital for Thoracic medicine, Tambaram, Institute of Child Health, Govt. Rajaji Hospital, Madurai, Govt. Vellore Medical College & hospital as well as non-government institution like St. John's hospital, Bangalore, Christian Medical college, Vellore and Asha Kirana hospital, Mysore Nilofer hospital, Hyderabad, etc.

Studies completed

(i) Study to evaluate the effect of physician's advice in quitting smoking in HIV and tuberculosis patients in south India- A pilot study

[Source of funding: Fogarty International, NIH]

BACKGROUND: Smoking is one of the major public health problems globally. Smokers who are infected with HIV develop additional risks, in addition to the general health consequences of smoking. It has been shown that there was a positive association between tobacco smoking and PTB and also higher morbidity, mortality and relapse rates in PTB smokers. It was proposed to study the efficacy of physician's advice in quitting smoking in patients with TB or HIV.

Aim : (i) to compare the effectiveness of Physician's advice using “modified 5 A” strategy to brochure and counselor's counseling in quitting smoking among patients with TB / or HIV

Methods : Patients with TB or HIV with history of current smoking were randomized to receive either Group A (Physician's advise + Counselors counseling + Brochure/educative material) or Group B (Counselors counseling + Brochure/educative material) strategy of smoking cessation, stratified based on nicotine dependence assessed using Fagerstrom dependence scale. Eighty smokers with HIV infection and 80 smokers with TB disease were enrolled to the study. In Group A, in addition to the administering of brochures and a standard counseling by counselor (strategy as in Group B), Physician's advise using 'modified 5 A' strategy was systematically approached in the five standard steps namely "Ask, Advise, Assess, Assist and Arrange". In addition, the physician delivered a brief structured advise to subject and his/her family member. Quitting rate at 1 month was assessed by questioning the patient and measuring urine nicotine levels.

Results: The study was started in August 2009 and completed in March 2012. Table 1.1 shows the baseline characters of subjects in the two disease groups. Physician's advice

had an efficacy of 30% while counseling alone had an efficacy of 10%.

Table 1.1 : Baseline characters of subjects in the two disease groups

Factor	TB (n=80)	HIV (n=80)
Age (years)		
≤ 29	8 (10.0)	9 (11.3)
30 – 40	32 (40.0)	48 (60.0)
41 – 50	30 (37.5)	19 (23.8)
>50	10 (12.5)	4 (5.0)
Education		
Illiterate	21 (26.3)	6 (7.5)
Primary	34 (42.5)	38 (47.5)
Secondary	24 (30.0)	31 (38.8)
Under Graduate	1 (1.3)	4 (5.0)
Post Graduate	-	1 (1.3)
Type of Smoking		
Cigarette	21 (26.3)	37 (46.3)
Bidi	23 (28.8)	14 (17.5)
Both	36 (45.0)	29 (36.3)
Age of Starting Smoking (years)		
≤ 10	14 (17.5)	8 (10.0)
11 – 15	20 (25.0)	17 (21.3)
16 – 20	32 (40.0)	36 (45.0)
>20	14 (17.5)	19 (23.8)
Duration of Smoking (years)		
≤ 10	7 (8.8)	9 (11.3)
11 – 20	24 (30.0)	42 (52.5)
21 – 30	27 (33.8)	21 (26.3)
>30	22 (27.5)	8 (10.0)
Tobacco Use		
≤ 10	36 (45.0)	37 (46.3)
11 – 20	25 (31.3)	28 (35.0)
>20	19 (23.8)	15 (18.8)
History of Alcohol		
Yes	67 (83.8)	61 (76.3)
No	13 (16.3)	19 (23.8)
Reason for Smoking*		
Pleasure	64 (80.0)	63 (78.8)
Family Tension	56 (70.0)	56 (70.0)
Work spot Tension	71 (88.8)	70 (87.5)
Others	6 (7.5)	9 (11.3)
Fagerstrom Score		
Low	48 (60.0)	56 (70.0)
High	32 (40.0)	24 (30.0)

Figures in parentheses indicate percentages

* Some subjects had multiple reasons

(Contact person: Dr. S. Ramesh Kumar, email: ramesh@nirt.res.in)

(ii) Anemia and nutrition among children with perinatally acquired HIV infection in south India

[Source of funding: ICMR Task Force]

BACKGROUND : Anemia is common in HIV infection and is a predictor of disease progression. In India 70 - 80 % of HIV positive children suffer from anemia. Previous studies done at St John's hospital and NIRT also showed that 60% and 51% of anemia prevalence in HIV children. Anemia has negative consequences on cognitive and physical development of children and its etiology is multi factorial- Nutritional (iron & micronutrient deficiency) as well as non-nutritional factors (anaemia of Inflammation). WHO guidelines state that low Hb may be managed with therapeutic Fe & folic acid supplementation for 3 months. However, in the absence of markers of iron deficiency anemia, Fe supplementation may add to existing Iron stores leading to iron overload.

Primary objective :

(i) to assess the prevalence of anemia and micronutrient deficiencies (iron, vitamins A, B12, and folic acid) among HIV-infected children in south India and also examine the nutritional and non-nutritional etiological factors contributing to anemia

Secondary objectives :

(a) to compare the effect of therapeutic iron supplementation in those with nutritional anemia and anemia of inflammation, using haematological endpoints (such as hemoglobin, markers of iron status) and measurable endpoints for HIV disease progression (CD4 counts, viral load, opportunistic infections, hospital admissions, death)

(b) to assess the effect of baseline anemia on growth and HIV disease progression status in children with HIV infection

Results: 240 children, (131 males, 54.6%) with perinatally acquired HIV infection aged between 2 and 12 years were recruited at three sites in south India. 80% of children were in WHO clinical stage 2, median CD4%, 25% (IQR=18, 33); and median CD4 count was 770 cells/mm3 (IQR=505, 1237). There was a high prevalence of malnutrition at baseline: proportion of children with stunting (height for age Z score HAZ< -2) was 40.0%; with underweight (weight for age Z score WAZ< -2) was 45.4%, and those with wasting (weight for height Z score WHZ< -2) was 23.3%, and children with low BMI (body mass index Z score BMIZ<-2) was 29.2%. 47% were anemic, with a mean Hb of 10.4gms/dl. Iron deficiency anemia was present in 86.2% of 239 children, vitamin A deficiency was present in 79% of 236 children and anemia due to chronic inflammation was seen in 59%.

Among children who received iron for 3 months, median Hb increased from 10.4 gm/dl to 10.9 mg/dl. Hemoglobin change was maximum after 1 year among children who received iron for 3-6 months, median Hb increased from 10.4 gm/dl to 11.3 mg/dl (p<0.05). The presence of iron did not independently affect growth or CD4 parameters; overall improvement of WAZ and HAZ were seen over 1 year irrespective of iron supplement. The prevalence of iron deficiency also decreased from 90% to 79% among those who received iron supplements. There was no change in the presence of chronic inflammation as measured by C-reactive protein among those who received iron supplements (Tables 1.2 & 1.3).

Table 1.2: Change in parameters from baseline to 1 year in the groups with and without iron supplements

Parameters		Received iron supplements (n=70)	No supplement received (n=42)	P-value
Hemoglobin	at baseline	10.4	12.0	0.003
	at 3 months	11.1	12.0	0.04
	at 1 year	11.3	11.9	0.2
Weight-for-age	at baseline	-2.2	-1.9	0.08
	at 1 year	-1.9	-1.8	0.2
Height-for-age	at baseline	-1.6	-1.5	0.2
	at 1 year	-1.3	-1.7	0.05
CD4%	at baseline	21.0	20.5	0.6
	at 1 year	26.5	26.0	0.7
Fe deficiency prevalence (StfR/logferritin>0.75)	at baseline	90.0	85.7	0.06
	at 3 months	79.2	80.0	1.0
Chronic inflammation (CRP>1)	at baseline	62.9	52.4	0.05
	at 3 months	57.9	40.0	0.06

Table 1.3: Comparison of hemoglobin change and disease progression among those children who were anemic or non-anemic at baseline

Parameter (median)	Anemic group median (q1, q3) (n=112)	Non- anemic group median (q1, q3) (n=128)	P value
Hemoglobin at 1 year (g/dl)	11.4 (10.6, 12.0)	12.1 (11.6,12.7)	< 0.0005
Weight-for-age Z score at 1 year	-1.9 (-3.0, -1.0)	-1.6 (-2.3, -0.7)	0.09
Height-for-age Z score at 1 year	-1.4 (-2.5, -0.8)	-1.3 (-2.1, -0.5)	0.2
CD4% at 1 year	26.0 (18.5, 35.0)	29.0(24.0, 36.0)	1
Serum iron level at 3 months (¼g/dL)	55.5 (36.8, 83.5)	69.0 (34.3,78.8)	0.05
Prevalence	n (%)	n (%)	
Iron deficiency at 3 months (n=74)	46 (79.3)	16 (92.0)	0.05
Severe clinical stage (Stage 3, 4) at 1 year (n=194)	10 (11.5)	3 (2.9)	0.02
Prevalence of malnutrition (BMI < -2.0) at 1 year (n=194)	23 (26.7)	23 (21.9)	0.4
Percentage of children anemic at 1 year (n=182)	43 (53.1)	20 (19.8)	< 0.0005

Conclusion: The prevalence of anemia-iron deficiency and vitamin A deficiency was high in this cohort of HIV-infected children, indicating that underlying micronutrient deficiencies were prominent. Independent risk factors for anemia were young age, presence of malnutrition, low CD4 counts and absence of ART. The combination of iron supplements and antiretroviral therapy appeared to provide the maximal benefit in Hb change, clinical and growth parameters. Despite supplementation, a substantial proportion of these children remained anemic after one year.

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Studies in progress:

(i) Randomized clinical trial to study the efficacy and tolerability of 3- and 4-month regimens containing moxifloxacin in the treatment of patients with sputum positive PTB

(CTRI Registration No.: PROVCTRI/2008/091/000024) [Source of funding: Intramural]

Background: The currently recommended 6-month regimen for the treatment of newly diagnosed PTB patients has been in use since the 1970s. This regimen, though highly effective, poses challenges for patients and providers due to the long duration. Shortening the duration of this treatment will therefore be an important contribution to TB control and is recognized as a research priority. To address this issue a randomized clinical trial is being conducted by the NIRT in Chennai and Madurai.

Aim : (i) in this trial, the standard 4-drug TB regimen is supplemented with moxifloxacin (MFX), a fluoroquiniolone with potent bactericidal and sterilising activities against M. tuberculosis, with the aim of shortening treatment duration**.**

Methods : Patients with newly diagnosed sputum positive, HIV sero-negative PTB are randomly allocated to 3-month or 4-month MFX regimens, or a 6-month control regimen (Table 1.4). Treatment is directly observed and response to treatment is assessed by clinical evaluations and with sputum examinations. The patients are also closely monitored for adverse drug reactions. Patients with successful treatment outcome are followed up for 24 months after completion of treatment with monthly evaluations for assessing recurrence of TB. The study regimens are described in table 1.4.

Table 1.4: Study regimens

Regimen	Intensive phase	Continuation phase	Duration (mths.)
Test regimen 1	3 RHZEM daily		3
Test regimen 2	2 RHZEM daily	2 RHM daily	4
Test regimen 3	2 RHZEM daily	2 RHM thrice weekly	4
Test regimen 4	2 RHZEM daily	2 RHEM thrice weekly	4
Control regimen	2 RHZE thrice weekly	4 RH thrice weekly	6

R rifampicin; H isoniazid; Z pyrazinamide; E ethambutol; M - moxifloxacin

Results: A total of 810 patients have been enrolled in the study. The baseline characteristics of these patients are shown in table 1.5. A majority of the patients were male, aged less than 40 years, and had advanced disease evidenced by strongly positive sputum cultures and radiological involvement of more than 2 lung zones.

Table 1.5: Baseline characteristics of 810 patients enrolled in study

Regimen	Test Reg. 1 (n = 112)	Test Reg. 2 (n = 173)	Test Reg. 3 (n = 177)	Test Reg. 4 (n = 175)	Control Reg. (n = 173)	Total (n = 810)
Sex						
Male	89 (79%)	133 (77%)	136 (77%)	118 (67%)	135 (78%)	611 (75%)
Age						
<40 years	77 (69%)	106 (61%)	122 (69%)	118 (67%)	112 (65%)	535 (66%)
Initial sputum culture						
2+ or 3+	109 (97%)	163 (95%)	166 (94%)	165 (96%)	169 (98%)	772 (95%)
Extent of Initial X-ray involvement (zones)						
> 2	87 (78%)	134 (78%)	141 (80%)	136 (78%)	135 (78%)	633 (78%)

A salient finding of this study is that the proportion of patients who became sputum culture negative after the initial 2 months of treatment was significantly higher (94%) in the MFX arm (consolidated for all four test regimens) compared to the control arm (77%). This observation which was made earlier (Annual Reports 2009-2010, 2010-2011, 2011-2012) is sustained even with the larger population. Fig. 1.1 illustrates the proportion of patients with negative sputum cultures at 15, 30, 45 and 60 days of treatment. This is a significant finding as it shows that patients treated with the MFX regimens become less infectious earlier and to a greater degree compared to those treated with the control regimen.

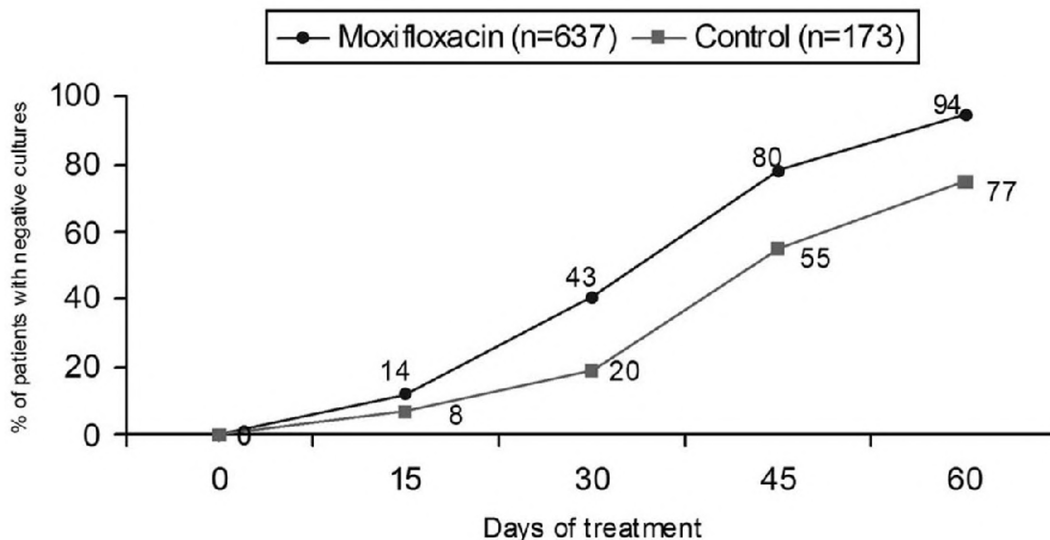

Fig. 1.1: Sputum culture conversion with treatment

Table 1.6 describes the results at the end of treatment in 711 patients, and recurrence of TB among those who had a favourable response at the end of treatment. Of patients treated with MFX regimens, 92 - 93% had negative sputum cultures at the end of treatment compared to 80% in the control regimen.

Of 637 patients with successful outcome at the end of treatment, 59 had recurrence of TB during post-treatment follow-up. TB recurrence was significantly higher in test regimen 1 (3-month MFX regimen) compared to the 4-month MFX regimens and the control regimen. Based on this information the Data and safety monitoring board (DSMB) had earlier recommended the temporary suspension of intake to this regimen (Annual Report 2010-2011), pending a more detailed review. Following an interim review of the data the DSMB has now recommended the cessation of recruitment to this regimen. Intake to the other regimens is continuing with a modified allocation ratio of 2:2:2:1.

Table 1.6: Response at the end of treatment and TB recurrence during follow-up

Regimen	Patients	Response at end of treatment			TB recurrence*
		Favorable	Unfavorable	Lost	
Test regimen 1	112	103 (92%)	2	7	20 (19%)
Test regimen 2	149	139 (93%)	5	5	10 (7%)
Test regimen 3	153	140 (92%)	6	7	13 (9%)
Test regimen 4	149	137 (92%)	6	6	9 (6%)
Control regimen	148	118 (80%)	12	18	7 (6%)

* in those with favourable response at the end of treatment (column 3)

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(ii) Randomized clinical trial to study the efficacy and tolerability of a 4-month regimen containing ofloxacin compared to the standard 6-month regimen in the treatment of patients with superficial lymphnode TB

(CTRI- Registration No: CTRI/2013/03/003481) [Source of funding: Intramural]

Background: TB lymphadenitis is the most common presentation of extra-pulmonary TB, accounting for 3040% of cases in reported series. Under the Revised National Tuberculosis Control Programme (RNTCP), patients with TB lymphadenitis are currently treated with a thrice weekly regimen (Category-I) with 4 drugs [Rifampicin (RMP), Isoniazid (INH), Ethambutol (EMB) and Pyrazinamide (PZA)] for the first two months followed by 2 drugs (RMP and INH) for the next four months. A study done at the NIRT has shown that even less intensive regimens, viz. a 6-month regimen of 2RHZ2/4RH2 and a 6-month regimen of RH daily were highly successful in patients with biopsy confirmed lymphnode TB in Madurai, south India. The delivery of TB chemotherapy in the field would be much easier if the duration of therapy could be shortened without sacrificing efficacy.

In the recent past the fluoroquinolone group of drugs has been demonstrated to have significant therapeutic potential in the management of TB. These bactericidal drugs, which inhibit DNA gyrase, are highly active against M. tuberculosis, including strains resistant to first line drugs. Fluoroquinolones are of particular interest, because there is no indication of cross-resistance to other antituberculous drugs.

In the current study we propose to investigate a regimem with 4-drugs [RMP, INH, PZA and Ofloxacin (OFX)] daily intensive phase of two months, followed by 3 drug (RMP, INH and OFX) thrice weekly continuation phase. The control regimen for comparison of outcome measures with the test regimens proposed for this study will be the standard 6-month thrice-weekly regimen of RMP, INH, EMB and PZA for 2 months followed by RMP and INH for 4 months.

Aims:

Primary aim : (i) to compare the efficacy of the regimens in terms of: (a) Response at the end of treatment (b) Relapse up to 24 months of follow-up after treatment in newly diagnosed superficial lymphnode TB patients treated with 4-month OFX containing regimens, with those treated with a 6-month regimen (control regimen).

Secondary aim : (i) to compare the incidence of (a) “Paradoxical reaction” during treatment and follow-up (b) Drug adverse reactions in newly diagnosed superficial lymphnode TB patients treated with 4-month OFX containing regimens, with those treated with a 6-month regimen (control regimen).

Method: The study intervention will consist of 2 regimens. In the test regimen OFX will be used along with RMP, INH and PZA and will be compared with a 6-month control regimen

Test regimen: RMP, INH, PZA and OFX daily for 2 months followed by RMP, INH and OFX thrice weekly for 2 months (2 RHZO7 / 2 RHO3) - duration 4 months.

Control regimen: Will consist of RMP, INH, EMB and PZA thrice weekly for 2 months followed by RMP and INH thrice weekly for 4 months (2 RHEZ 3 / 4 RH3) - duration 6 months.

Patients attending the surgical, medical clinics of Madurai Rajaji Hospital, Govt. Stanley Hospital, Govt. General Hospital and Govt. Hospitals and Corporation RNTCP Centres in Chennai with fine needle aspiration cytology (FNAC) proved superficial TB lymphadenitis or clinical evidence of lymphnode enlargement will be considered for the study. Biopsies will be done for all patients.

Study outcome will be

a) Favorable or unfavorable response at the end of treatment

b) Relapse during follow-up in those with favorable response at the end of treatment

Secondary outcome measures will be paradoxical reactions and adverse reactions to anti-TB drugs.

The study is being conducted in Chennai and Madurai. The estimated sample size for this trial is 420 patients; so far 15 patients have been enrolled to the pilot study. The study is ongoing.

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(iii) Randomized controlled clinical trial comparing daily vs. intermittent 6 month short course chemotherapy in reducing failures & Emergence of Acquired Rifampicin Resistance in patients with HIV and PTB

(CTRI Registration No: 476/09, NCT No. 933790) [Source of funding: USAID, ICMR]

Background: HIV-TB is an important dual infection in India demanding attention from programme managers and clinicians. Anti-TB treatment (ATT), both duration as well as frequency of administration, have been major issues in research involving patients with dual infection due to complexity of drug and disease interaction, overlapping toxicities as well as high failure and mortality rates reported by various studies.

Aim : (i) to compare daily vs. intermittent therapy of ATT is designed to compare various aspects of TB outcome in HIV-PTB such as sputum conversion, immune reconstitution inflammatory syndrome (IRIS), emergence of acquired rifampicin resistance (ARR), radiological improvement, pharmacokinetics of ATT drugs and toxicity profile with respect to dosing schedule

Methods : HIV-TB patients with culture positive TB are randomized to three regimens viz.:

(1) daily regimen (2EHRZ7/4HR7), (2) part daily (2EHRZ7/4HR3) and (3) a fully intermittent regimen (2EHRZ3 /4HR3), given for 6 months duration and followed up for a further period of one year, stratification based on CD4 and sputum smear grading. Blood samples at 2-hr post dosing is being collected at months 1 and 5 of ATT. Plasma RMP and INH estimations are undertaken by HPLC in a blinded manner. Toxicity was monitored using modified CTC and DAIDS criteria. Unfavourable responses in each regimen during treatment and follow-up are compared. Both intent to treat analysis and perprotocol analysis will be performed.

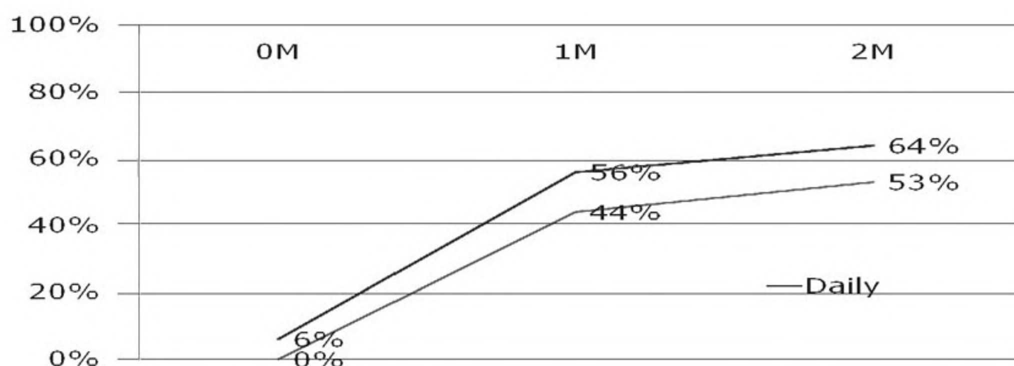
Results : The sample size is 420. As on 31.03.13, 181 patients (46 from Madurai and 135 from Chennai) have been randomized to the three regimens (daily-62, part daily-60, intermittent-59). Mean age and weight of the study participants was 39 years and 43 kgs respectively. The median CD4 is 142 cells /mm3 (IQR-73-331) and the median viral load is 5.2 copies/ml (IQR-3.9-5.8) indicative of the advanced stage of HIV. Two-third of patients has a lower level of RMP below the therapeutic range, irrespective of the regimen. Study is ongoing.

For analysis, the daily regimen and the part daily regimen were combined (being identical) and the sputum conversion in the first 2 months is provided below. The patients had comparable characteristics with similar involvement in chest X-ray, initial sputum grading and time to initiation of ART in both the groups. The following table (table 1.7) shows the baseline demographics of patients in the trial.

Table 1.7: Baseline demographics of patients

Baseline demographics	n=181
Mean age ±SD (years)	38±9
Mean weight +SD (kgs)	43.1±7.6
Male : Female ratio	135:46
Median CD4(IQR) (cells/mm3)	142 (73-331)
Median VL (IQR) ($\log_{10}$ copies/ml)	5.2 (3.9-5.8)

Sputum smear conversion



NIRT - ICMR

Fig.1.2: Sputum smear conversion

The smear conversion was higher in the daily regimen than in the intermittent regimen but did not achieve statistical significance (Fig. 1.2). However, the culture conversion was significantly higher in the daily regimen (Fig. 1.3). It remains to be seen if this has an influence over the final outcome of treatment after 6 months of ATT.

Sputum Culture conversion between daily and intermittent region

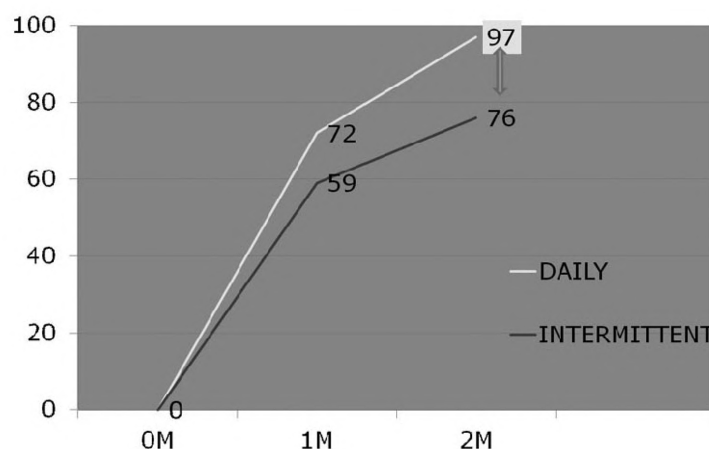


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Fig.1.3: Sputum culture conversion

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(iv) Evaluation of newer diagnostic tools and feasibility of consensus case definition in the diagnosis of intra-thoracic TB in children

[Source of funding: USAID]

Background: The lack of a gold standard for diagnosis is a major obstacle to accurately quantifying the true burden of childhood TB which is probably both over and under-diagnosed among children in different settings. The need for improved TB diagnostics in children is consistently acknowledged. Promising novel techniques (Xpert® MTB/RIF, urine LAM) that have been developed for the diagnosis of TB need to be tested and validated in children. Xpert® MTB/RIF (Cepheid, Sunnyvale, USA) is an automated user-friendly real-time polymerase chain reaction (RT-PCR) assay designed for the rapid and simultaneous detection of M. tuberculosis (M.tb) and RMP resistance. Lipoarabinimannan (LAM) is a structurally important 17.5kD heat-stable glycolipid found in the cell wall of M.tb. Detection of LAM antigens in urine has several potential advantages as urine samples are simple to collect and process.

A group of international experts have developed a consensus reference standard and case definition for PTB in children, for use in research and clinical settings. This study will provide an ideal opportunity to test the feasibility and clinical relevance of this consensus case definition.

Aims: (i) to determine the diagnostic accuracy of Xpert® MTB/RIF (Cepheid, Sunnyvale, USA) in the diagnosis of intra-thoracic TB in children and to study the feasibility of utilizing the newly developed consensus case definition

Secondary objective of the study is to compare the yield of M.tb from different specimen collection methods (expectorated / induced sputum, gastric lavage in stool etc.) in various age groups and to evaluate urine LAM, in the diagnosis of intra-thoracic TB.

Methods: All children aged < 15 yrs attending the pediatric out-patient department in the Institute of Child Health, Govt. Stanley Hospital, Kilpauk Medical College Hospital, Chennai Corporation dispensaries in Chennai, Govt. Hospital for Thoracic Medicine, Tambaram, Christian Medical College and Govt. Medical College, Vellore and Govt. Rajaji Hospital, Madurai with any of the following will be screened for the study- in the presence of (a) cough (b) weight loss/ failure to thrive (c) persistent unexplained fever (d) persistent, unexplained lethargy or reduced playfulness. Symptom screening, a detailed general and clinical evaluation will be done. Chest X-ray, tuberculin skin test (TST), collection of gastric lavage/induced/expectorated sputum for Xpert® MTB/RIF, acid fast bacilli (AFB) smear, culture and DST if culture positive, will be done.

In addition, in infants (ie.aged < 1yr), stools will be collected for 2 consecutive days which will be examined by Xpert® MTB/RIF for AFB smear, culture and DST if culture positive. Urine for LAM, blood investigations and FNAC will be done if needed. TB diagnosis in children will be made and classified into the following groups based on smear result, chest radiograph and TST as confirmed TB, probable TB and others. Follow-up will be done at 2 weeks, 4 weeks, 8 weeks, and end of treatment. The pilot study has been initiated and as of now 50 children have been recruited.

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(v) Predictors and immunologic characterization of TB-associated IRIS in HIV-TB patients started on antiretroviral therapy

[Source of funding: National Institute of Health and ICMR]

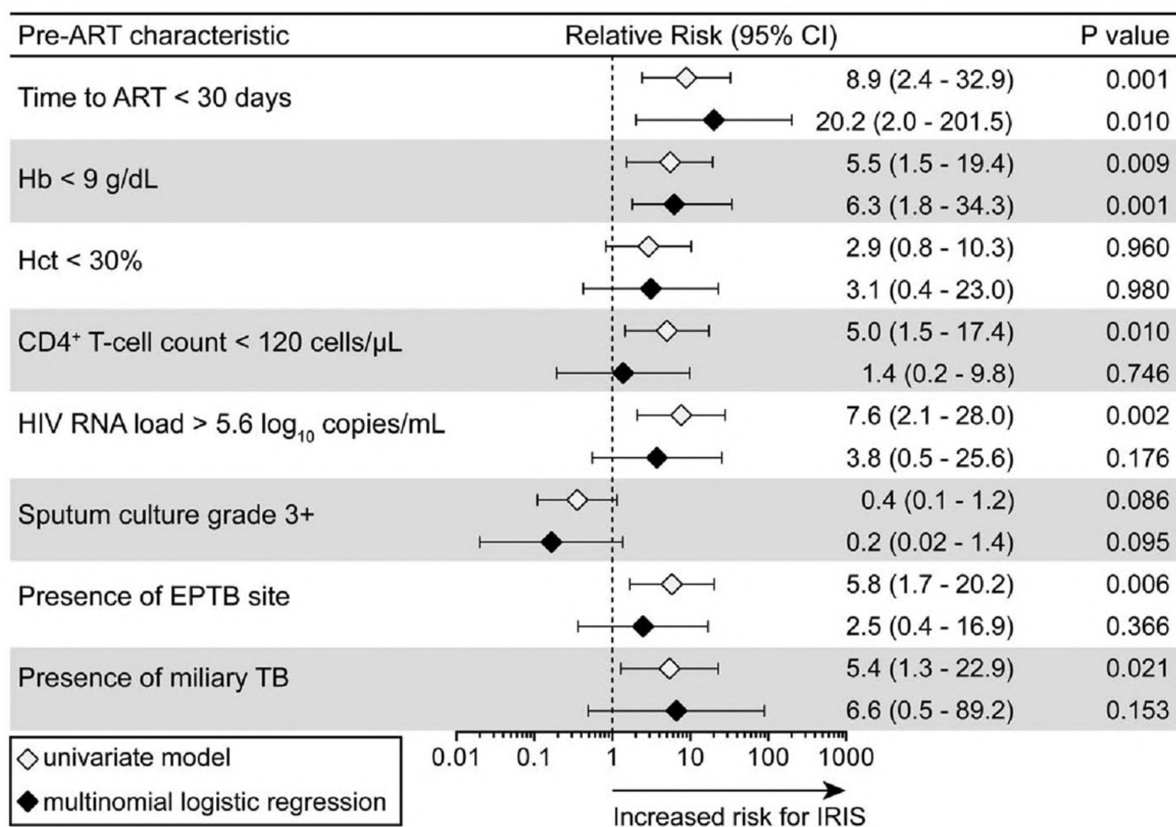
Background: IRIS or Immune restoration disease is the paradoxical worsening or unmasking of infection, tumor-associated pathology, or autoimmune disease in HIV-infected patients after starting antiretroviral therapy.

Aim: (i) to evaluate early predictors which could help foretell the syndrome and minimize the morbidity and mortality associated with it

Methods : HIV/TB co-infected patients with and without IRIS manifestations were prospectively followed through progressive stages of immune restoration and clinical and lab predictors were evaluated. Confirmation of an IRIS diagnosis was done by International network for study of HIV associated IRIS criteria that would assist in timely administration of corticosteroids or other anti-inflammatory therapy to reduce the mortality and morbidity associated with this syndrome.

Results : A total of 48 HIV positive patients with newly diagnosed culture confirmed PTB (45 males: 12 females), who were ATT and ART naïve, were enrolled. The two groups were similar with respect to gender, age, body weight and CD8+ T-cell counts and levels of alanine transminase and aspartate transaminase at baseline. Univariate analysis showed that low CD4, high viral load, opportunistic infection, miliary TB, presence of extra pulmonary focus, low HB and shorter ATT-ART interval were all associated with IRIS. Hb and time interval between ATT-ART remained significant in multivariate regression along with IL-6 and C-reactive protein. The study is ongoing. Table 1.8 shows the relative risk of baseline pre-ART clinical and laboratory factors in triggering IRIS.

Table 1.8: Relative risk of baseline pre-anti retroviral therapy



Pre-ART characteristic	Relative Risk (95% CI)	P value
Time to ART < 30 days	◇ 8.9 (2.4 - 32.9)	0.001
	◆ 20.2 (2.0 - 201.5)	0.010
Hb < 9 g/dL	◇ 5.5 (1.5 - 19.4)	0.009
	◆ 6.3 (1.8 - 34.3)	0.001
Hct < 30%	◇ 2.9 (0.8 - 10.3)	0.960
	◆ 3.1 (0.4 - 23.0)	0.980
$CD4^+$ T-cell count < 120 cells/µL	◇ 5.0 (1.5 - 17.4)	0.010
	◆ 1.4 (0.2 - 9.8)	0.746
HIV RNA load > 5.6 log_{10} copies/mL	◇ 7.6 (2.1 - 28.0)	0.002
	◆ 3.8 (0.5 - 25.6)	0.176
Sputum culture grade 3+	◇ 0.4 (0.1 - 1.2)	0.086
	◆ 0.2 (0.02 - 1.4)	0.095
Presence of EPTB site	◇ 5.8 (1.7 - 20.2)	0.006
	◆ 2.5 (0.4 - 16.9)	0.366
Presence of miliary TB	◇ 5.4 (1.3 - 22.9)	0.021
	◆ 6.6 (0.5 - 89.2)	0.153

The table shows the pre-ART characteristics (both clinical and lab) with subsequent IRIS events. The unadjusted univariate analysis showed that shorter time to ART, lower CD4, presence of EPTB and miliary TB, higher plasma HIV RNA copies and lower HB to be significant risk factors for the occurrence of IRIS. ART initiation and HB at baseline remained significant in the multiple logistic regression, possibly due to smaller sample that was being studied. Adjustment was performed for all variables presented and also included age and gender. 95% CI, 95% confidence interval.

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(vi) Study on the effectiveness and feasibility of TB preventive therapy for people living with HIV in India - adults and children

[Source of funding: USAID]

Background : HIV is the strongest risk factor for developing TB disease in those with latent or new M. tuberculosis infection. In response to the dual epidemics of HIV and TB, WHO currently recommends that all HIV-infected persons be screened for TB and HIV-infected persons without active TB disease, should be evaluated for treatment of latent TB (LTB) infection, also known as isoniazid TB preventive therapy (IPT). The WHO has recently published guidance on IPT for both children and adults, mainly based on adult data. Hence people living with HIV (PLHIV) both adults and children should routinely be screened for TB as a part of standard clinical care, whether they are receiving TB prophylaxis or ART.

Aim : (i) is a prospective multicentric study with phased implementation, looking at the feasibility and effectiveness of TB preventive therapy for HIV- infected adults and children attending ART centers in several states

Primary aim of the study is to assess the effectiveness of IPT in PLHIV (at different CD4 counts and both pre-ART and on ART).

Secondary aims of the study are:

- to assess the effectiveness of simple algorithms to exclude active TB prior to IPT initiation
- to assess the feasibility of providing IPT for PLHIV attending ART centers
- to measure number needed to screen and number needed to treat to prevent one case of TB

Methods : The study consists of two phase

Phase I: Enhanced TB surveillance (all sites, all ART centre attendees). In this phase documentation of TB screening will be strengthened along with the current existing procedures of screening and co-trimoxazole prophylaxis for all HIV-infected adults and children. On entry patients will be offered a standardized TB symptom screen. Phase I will be conducted for 6 months from the time the study is initiated, at each ART centre. The outcome for Phase I will be the incidence of TB at that ART centre during this period.

Phase II: Provision of IPT: After 6 months (following the completion of Phase I), Phase II will begin at that ART centre and all eligible patients attending that ART centre will be administered IPT.

Results : Study was initiated in Phased manner at 5 ART centers in Tamilnadu, two in Karnataka, one each in Andhra Pradesh and New Delhi. As on 31st March 2013, 2200 adults and 400 children have been recruited to the study. The sample size is 6000 adults and 1800 HIV-infected children and the study is ongoing.

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(vii) High density lipoprotein cholesterol and gene polymorphisms among HIV-infected south Indians on first line ART

[Source of funding: Fogarty International Centre (NIH)]

Background : The introduction of generic combination ART for the treatment of HIV infection has dramatically modified the natural history of HIV infection, leading to a significant reduction in morbidity and mortality as well as a better quality of life. Unfortunately, the long-term toxicity from surviving with HIV infection and ART, especially metabolic and bone changes, insulin resistance etc. is becoming more and more recognized. Even prior to the availability of ART, HIV-infected individuals were noted to have metabolic abnormalities like increased triglycerides (TGL) and decreased HDL. However, not all patients show an increase in HDL-c while on NNRTI based antiretroviral regimens, despite similar exposure to drugs, comparable demographic and other characteristics. The reasons for this discrepancy may be related to host genetic factors.

Aim : (i) is a cross-sectional study to look at specific gene polymorphism and change in HDL-cholesterol levels, among HIV-infected patients. We plan to determine whether HDL-cholesterol gene polymorphisms (single nucleotide polymorphisms in ABCA1, CETP, LIPC, LPL and APOC3 genes) are associated with unfavorable blood HDL- cholesterol levels, in HIV-infected adults in south India, after 12-15 months of standard ART

Methods : HIV-infected adults (18 60 years) on WHO/NACO recommended first-line ART regimen with nevirapine (NVP) and 2 NRTI drugs, having completed at least 12 months and not exceeding 15 months of NVP based ART regimen, are the study participants. Patients who are seriously ill, on efavirenz (EFV) based ART, have had ART changed or interrupted for more than a month continuously any time during the last 18 months or on II line ART will not be considered for the study.

Demographic details, past medical history and drug history including details of antiretroviral therapy, CD4, heamoglobin, weight and height at the time of ART initiation will be collected from their ART records. Current height, weight, midarm, waist and hip circumferences along with blood pressure will be recorded. Approximately 7ml of blood will be drawn for fasting lipids, glucose, and gene polymorphism studies. The study outcome will be to look for the presence of single nucleotide polymorphism in ABCA1, CETP, LIPC, LPL and APOC3 genes, in individuals with low HDL-c after 12 15 months of NVP based first line ART.

The study was initiated in January 2013. As on 31st March 2013, 10 patients have been recruited to the study. The sample size is 300. Study is ongoing.

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(viii) HIV-associated lipodystrophy syndrome in children: Role of nutrition, ART and genes (5RO1 A1084390)

[Source of funding: National Institute of Health]

Background : The incidence and pattern of metabolic complications among HIV-infected children who are started on Stavudine based ART are not known. Specifically, the role of genetic factors (single nucleotide polymorphisms in APOA5, APOC3, APOE, CETP and PLIN genes) in the development of metabolic complications, namely fat redistribution, insulin resistance and dyslipidemia are not well documented in India. Various risk factors

and lipid gene polymorphism will be correlated with lipodystrophy, lipoatrophy, dyslipidemia and insulin resistance. The study will provide information, not currently available; on the metabolic complication of HIV and ART, in children who take Stavudine containing antiretroviral treatment regimen. This knowledge of risk factors will be crucial to physicians and policy makers to design better treatment strategies for children.

Aim : (i) this is a prospective multi-centric study observational undertaken at NIRT, Chennai and Madurai, St. John's National Academy of Health Sciences, and Indira Gandhi Hospital, Bangalore, to determine the incidence and risk factors for dyslipidemia, abnormalities in glucose tolerance and body shape abnormalities, in HIV-infected children between the ages of 2 and 12 years at 12 months after initiating ART as well as to determine the role of genetic factors in the development of fat redistribution, insulin resistance and dyslipidemia

Methods : HIV-infected children who are about to initiate ART are recruited. Details about their demographics, clinical and dietary history (food security questionnaire, 24 hr dietary recall), physical examination, including anthropometric measurements are collected at baseline. They are followed every 3 months upto 12 months after initiation of ART. Blood investigations to measure lipid profile, peripheral insulin resistance, C-reactive protein, hematology, CD4 cell counts and viral load measurements are done at baseline, 6th & 12th month. The following single nucleotide polymorphisms will be studied:

APOA5 (64G>C and -1131T>C), APOC3 (-482 C>T, -455 T>C and 3238 C>G), APOE (E2, E4 alleles), CETP (279 G>A, -629 C>A) and PLIN (6209T>C, 11482G>A, 13041A>G and 14995A>T).

Results : The study was initiated in June 2011. As on 31st March 2013, 140 children between the age group of 2-12 years have been recruited to the study. The sample size is 440 (220 at each site) and the study is ongoing.

(Contact person: Dr. Soumya Swaminathan, email: soumyas@nirt.res.in)

(ix) Community volunteers, solidarity and case management of TB

[Source of funding: NIH RO1 No. 5RO1HD058831]

Background & Aim : The objective of the project is to compare the efficacy of using directly observed treatment (DOT) providers from the patient's community or neighbourhood and compare them with government DOT providers for the case management of TB. The secondary objective is to establish the situation where these community volunteers will be effective.

Methods: The study is ongoing in the 3 TUs (Arakonam, Natramppalli and Wallajah) of Vellore District. Two lakh and eighty thousand households have to be surveyed to get a sample of 3000 patients to randomize into 3 groups of directly observed treatment short-couse (DOTS) providers. So far 80,000 households are surveyed.

(Contact person: Dr Pradeep A Menon; email: pradeepamenon@nirt.res.in)

2
LABORATORY STUDIES
Department of Bacteriology

Overview: Department of Bacteriology has been supporting controlled clinical trials and projects conducted by the NIRT. Routine activities of the lab include diagnosis of TB by smear microscopy, culture and DST of MTB for patients under clinical trials and outside referral cases. In addition, the lab performs drug resistance surveillance (DRS) studies for estimating the prevalence of resistance to first line and second line drugs in the community. The department is also involved in basic and applied research on mycobacteriology. Screening of natural and synthetic compounds for antitubercular activity and optimizing LRP for rapid diagnosis and DST of MTB are some of ongoing activities. The laboratory functions as a National Reference Laboratory under the RNTCP and as a Supranational Reference Laboratory under WHO extends its technical support to ten IRLs, National reference laboratories in India and NRLs of SAARC countries. The support is in the form of training the program personnel, ensuring external quality assurance in sputum microscopy, inspection and certification of laboratories for performing culture and DST to first line and second line drugs through proficiency testing. Line Probe Assay (LPA) for rapid detection of resistance to RMP and INH directly from sputum samples was established in the department through coordinated efforts by NIRT, WHO SEARO Delhi and FIND India.

Studies Completed :

(i) TB drug resistance surveillance in the state of Tamil Nadu, India

[Source of funding: USAID through WHO, SEARO, New Delhi]

Background: Surveillance of TB drug resistance was previously carried out in a few states of India viz. Tamil Nadu, Gujarat, Maharastra and Andhra Pradesh. The **c**ontinuous surveillance of drug resistance will provide information which will serve as a useful parameter in the evaluation of the TB control programme. Hence, it was proposed to conduct a population-based survey of anti-TB drug resistance in the state of Tamil Nadu.

Aim : (i) to determine the proportion of drug resistance in new and in previously treated cases of PTB and to use the level of drug resistance as a performance indicator for the RNTCP

Methods : Based on cluster sampling, the sample size calculated for new sputum smear positive cases (NSP) and for re-treatment (RT) cases were 1680 and 992, respectively. A random of 70 designated microscopic centers (DMCs) was selected from the list of 700 DMCs by adopting population proportion to size (PPS) method. Culture and susceptibility testing were done for the isolates using standard methods. The economic variant of proportion sensitivity test was performed for two first line drugs**,** namely**,** INH and RMP and for two second line drugs, namely, OFX and kanamycin, for the simultaneous detection of MDR as well as XDR-TB.

Results : A total of 1524 and 902 patients were enrolled respectively for NSP and RT cases. The prevalence of MDR-TB among NSP and RT cases was 1.8 and 13.2% respectively (Table 2.1). Considering the RMP mono-resistance as a surrogate marker for MDR-TB the level of MDR-TB was 2.6% and 15.1% among NSP and RT cases respectively. Further, the prevalence of XDR-TB among RT cases was 0.6% and none of the isolates among NSP cases showed XDR-TB.

Table 2.1: The prevalence of first line and second line drug resistance in *M. tuberculosis* isolates among new and retreated cases of TB

	New cases (n=1220)		Re-treated cases (n=714)	
	Numbers (%)	CI	Numbers (%)	CI
Susceptible to all drugs	976 (80.0)	77.8 - 82.2	444 (62.1)	58.5 - 65.7
Any Resistance				
Isoniazid	127 (10.4)	8.7 - 12.1	214 (30)	26.7 – 33.4
Rifampicin	32 (2.6)	1.7 – 3.5	108 (15.1)	12.6 – 17.9
Kanamycin	1 (0.1)	0 – 0.2	7 (1)	0.3 – 1.9
Ofloxacin	127 (10.4)	8.7 – 12.1	99 (13.9)	11.4 – 16.5
Mono Resistance				
Isoniazid	91 (7.5)	6.0 – 8.9	95 (13.3)	10.8 – 15.8
Rifampicin	8 (0.7)	0.2 – 1.1	11 (1.5)	0.6 – 2.4
Kanamycin	0	0	1 (0.1)	0 – 0.4
Ofloxacin	107 (8.8)	7.2 – 10.4	41 (5.7)	4.0 – 7.4
MDR	22 (1.8)	1.1 – 2.5	94 (13.2)	10.8 – 15.8
Pre- XDR	5 (0.4)	0.1 – 0.8	28 (3.9)	2.5 – 5.3
XDR	0	0	4 (0.6)	0.1 - 1.3

Studies in progress:

(i) Viability and retrieval of *M. tuberculosis* from Kirchner's medium using bovine albumin serum instead of fetal calf serum

Background : Mitchison *et al.,* (2003) used Kirchner's medium with Polymyxin B, Amphotericin B, Carbenicillin and Trimethoprim for the isolation of mycobacteria from clinical extra pulmonary specimens. Results obtained were promising and it was considered as an alternative media for the isolation of extra pulmonary specimens.

Aims : (i) to substitute bovine serum albumin (BSA) for fetal calf serum (FCS) in Kirchner's medium for the isolation and recovery of *M. tuberculosis* from extra pulmonary specimens

(ii) to compare growth and retrieval of *M. tuberculosis* from Kirchner's medium using FCS and BSA

Methods : Three hundred and three extra pulmonary specimens received at NIRT were used in this study. The specimens were processed with 5% H_2S0_4 and inoculated into A set of Lowenstein Jensen medium (LJ), LJ-SP (LJ with sodium pyruvate), one selective Krichner's medium (SKM) and one BSA medium. Every week reading was taken, up to 6 weeks. After 6th week reading, SKM and BSA was processed by modified Petroff's method and inoculated on to LJ medium. LJ was read up to 8 weeks.

Results: Results are available for 200 sampels and awaited for 103 samples. The weighted kappa showed that the BSA medium has good agreement with the LJ method than the SKM. The sensitivity (62.5%) and specificity (99.24%) were at their maximum on the BSA medium which also compared through receiver operating curve (0.809**).

Conclusion : Kirchner's medium supplemented with BSA was comparable to FCS and the former was found to be slightly better than FCS in the recovery of *M. tuberculosis*. BSA can be used as an effective alternative to FCS for preparing Kirchner's liquid medium as it yields rapid growth of MTB with clear morphology with less or little contamination. Cost effectiveness, commercial availability, ease of storage, lack of contamination makes BSA a more ideal enrichment source for use in Kirchner's medium.

(Contact person: Dr. Gomathi Sekar, e.mail: gomathis@nirt.res.in)

(ii) Modified 'pot method' of staining AFB using phenol ammonium sulphate basic fuchsin tablets

Background : TB diagnosis mainly relies on direct sputum microscopy by Ziehl-Neelsen (ZN) staining method which is implemented in primary health centers by National TB Programmes in high TB burden countries.

Improper handling of specimen while making smears and disposal of the specimen leads to formation of aerosols. Therefore it is essential to reduce further risks of laboratory acquired infection. In order to overcome the above limitations, Selvakumar *et al.,* developed a 'pot-method' of staining AFB in sputum samples in sputum containers. However, in the pot-method' the usage of liquid form of phenol ammonium sulphate basic fuchsin solution (PhAS) has certain disadvantages.The preparation and distribution of the solution to various centers is challenging in the field. Vels University, Pallavaram, Chennai has signed an MOU with NIRT Chennai to prepare the tablets of PhAS for 'pot-method'.

Aim : (i) to evaluate the efficacy of tablets to stain AFB in sputum by carrying out suitable experiments at NIRT

Method : Excess sputum from patients in NIRT clinical trials will be used for this study at different time point to standardize the PhAS tablets. Three different methods were tested with the given tablets:

(i) The tablet was mixed with the processed sputum deposits. Smears were made at 15mts, 30 mts, 1 hr, 2 hrs and 3 hrs. The smears were air dried, heat fixed and decolorized with 25% acid alcohol for 2-3 mts, followed by counter staining with 0.1% methylene blue for 30-40 seconds. The results with PhAS were not comparable with original smear results.

(ii) When the tablet was used in direct sputum, the tablet did not mix in sputum.

(iii) In the next batch, tablet was used in sputum but it was dissolved after 48 hrs. In this case, AFB could not be observed but resulted in a blue background. When 0.5ml of 70% ethanol was added, AFB was seen under the microscope.

Results: Once standardization is completed, the smears will be read according to the RNTCP guidelines. The results will be analyzed against culture as the gold standard.

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(iii) Study to evaluate baseline anti-TB and anti-HIV properties of transitmycin from marine *Streptomyces* sp.

Antitubercular property of transitmycin, a novel antibiotic from marine *Streptomyces* sp, isolated from sea sediment off Rameswaram coast, was tested against standard strains and clinical isolates of *M. tuberculosis* and HIV. All the 100 *M. tuberculosis* isolates were inhibited at 10µg/ml concentrations while all the 20 HIV isolates were inhibited at 0.1 µg/ ml concentration. Transitmycin was completely soluble in various organic solvents such as methanol, acetone, ethyl acetate, chloroform, dichloromethane, diethyl ether, partially soluble in water and insoluble in n-hexane. Transitmycin was stable at all the tested temperature ranging from 4°C80^{o}C. Dose response of Tr was tested against *M. tuberculosis* and compared with known anti-TB drugs viz. INH and RMP. Results indicated that compared to INH, Tr showed better killing at 1uM concentration; compared to Rif killing effect of Tr was equivalent at 10uM concentration. Transitmycin inhibited 16 out of 18 MDR-TB isolates at 5µg/ml concentration. Further animal toxicity studies and studies of cyto and genotoxicity will be undertaken.

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(**iv) Screening for potent proteasome inhibitors of *M. tuberculosis***

Background : Proteasome is important during the dormant phase of *M. tuberculosis*, making it an attractive target for compounds that may interfere with dormant bacilli. However, due to the inherent cytotoxicity of proteasome inhibitors, chemical compounds targeting the *M. tuberculosis* proteasome must exhibit high selectivity for *M. tuberculosis* over human proteasomes in order to be considered for development as chemotherapeutics for TB.

Aims : (i) the proposed work aims at expressing the bacterial proteasome from different strains of *M. tuberculosis* and to compare the levels of expression in them. Potent proteasome inhibitors are to be identified among the antitubercular compounds from marine actinomycetes, and plant extracts with minimal/no activity against human proteasome with proteasomes expressed from different strains of *M. tuberculosis*. Using bio-informative tools the effect of the existing and newly identified anti-TB drugs on bacterial proteasome and human proteasome are to be elucidated

Results : RT-PCR technique is used for analyzing gene expression levels in active and dormant strains.Active strains of variable anti-microbial susceptibility (XDR, MDR, SHRE) were retrieved from the inoculated LJ slopes. RNA from both active and dormant strains for XDR, MDR and SHRE were isolated using guanidine isothiocynate method. The extracted RNA was first reverse transcribed into cDNA. The resulting cDNA was used as templates for subsequent PCR amplification using primers specific for *PrcA, PrcB* and 16srRNA genes. The following Fig. 2.1 represents amplified product of various anti-microbial susceptibility strains in active condition and the following Fig. 2.2 represents amplified product of various strains in dormant condition.

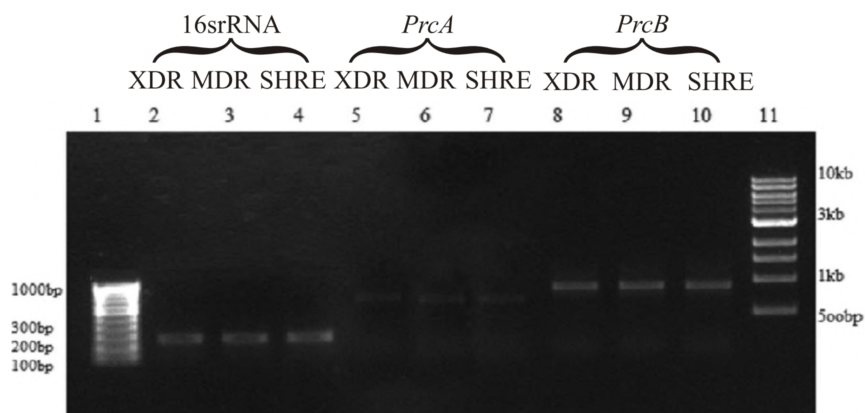

Fig. 2.1: Antimicrobial susceptibility strains in active conditions

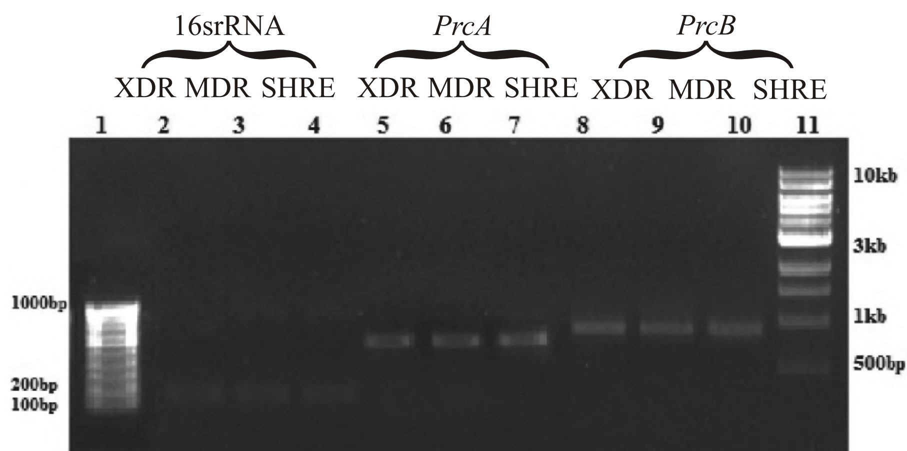

Fig. 2.2: Antimicrobial susceptibility strains in dormant conditions

Proteasome genes (*PrcA* and *PrcB*) got expressed in both active and dormant strains of various anti-microbial susceptibility (XDR, MDR, SHRE). We have used 16srRNA as housekeeping gene to compare the expression of proteasome genes.

In case of active strains, *PrcB* gene shows more expression than *PrcA* and more or less equivalent expression with 16srRNA gene as shown in the Fig. 2.3 (left side). While in case of dormant strains, *PrcA* and *PrcB* gene collectively shows more expression than 16srRNA gene which is represented in the Fig. 2.3 (right side). Hence the proteasome gene expression is found to be higher than the house keeping gene in case of hypoxic stress induced strains confirming proteasomes to be a potential drug target in dormant condition.

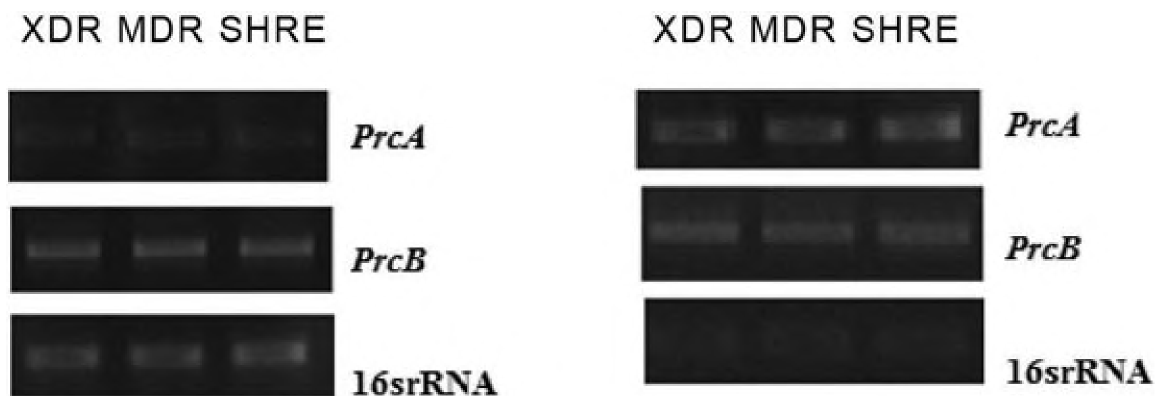

Fig. 2.3: A - Active strains B - Dormant strains

Cloning both prcA and B in a single vector followed by expression and purification of the protein has to be outsourced and the process has been initiated.

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(v) Rapid detection of RMP and INH drug susceptibility using LRP assay

Background : Emergence of MDR and XDR-TB has emphasized the need for rapidly determining the DST of RMP and INH. Egg based and liquid based (MGIT) methods require 28 days and 5 days. LRP assay can report DST for first line in 72 hrs and in this report we report the possibility for still shortening the time period for diagnosing MDR-TB.

Aim : (i) to determine Rifampicin (R) and Isoniazid (I) DST using LRP assay within 24 hrs

Methods : A total of 105 primary cultures of *M. tuberculosis* clinical isolates for RMP and 84 for INH were used for determining the DST using LRP with an incubation time of 4 hrs. Based on the results of this experiment another set of fifteen clinical isolates were used to determine DST for INH with a total incubation time of 24 hrs. The results were compared with the minimal inhibitory concentration method using solid based conventional method as gold standard. Suspension of *M. tuberculosis* cultures of 1 Mcfarland standard was prepared from LJ slopes and was incubated with drugs RMP (2μg/ml) and INH (0.2μg/ml) in separate vials along with controls without drugs. LRP construct phAETRC202 was added immediately for R and after one hr and 17 hrs later for I. After an incubation of 4 hrs 100μl of phage cell mixture with and without drug was mixed with equal volume of D-luciferin and the relative light units (RLU) were measured in luminometer. Percentage reduction in RLU of the test compared to control was calculated using the formula,

% Reduction in RLU = Control RLU Test RLU / Control RLU X 100

Samples showing 50% or less reduction in RLU are considered resistant (1 50% resistant; 51 100% susceptible).

Results: RMP susceptibility using LRP assay showed a specificity of 96.5% and sensitivity of 100% when compared with the conventional solid based method when the drug was added simultaneously along with the LRP. The results were available on the same day. INH susceptibility testing yielded a sensitivity of 75% and specificity of 18.5% when the phages were added after one hr after the drug exposure. When LRP was added 17 hrs post drug exposure 13 out of 15 samples were found to be INH susceptible and rest were resistant

Discussion: Mode of action of R and I on *M. tuberculosis* are different. Since target for R is on the RNA polymerase and I act on the fatty acid synthase, DST for R does not require an incubation period, whereas INH requires. Hence we tried a period of one hr in the case of I, but it seems to be insufficient (sensitivity and specificity were 75% and 18%) and the results shows that prolonged exposure to I is required. When the cells were exposed to the drug (INH) for an overnight period, sensitivity was 100% and specificity was 40%. But this was done with a very low sample number of 15 cultures and hence the specificity was too low and to get a real picture a large sample size is required. But in the case of R the results shows a very good correlation with that of the conventional methodology with same day and overnight formats.

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Dept. of Biochemistry & Clinical Pharmacology

Overview: The Department of Biochemistry & Clinical Pharmacology offers support to the controlled clinical trials by way of clinical biochemistry testing and checking the quality of drugs before administering to patients admitted to the various controlled clinical trials. In the past, extensive work was carried out to determine INH acetylator phenotyping using blood, urine and saliva. The mechanism of development of toxicity to RMP, INH and PZA was investigated. It was established that non-invasive method of estimating salivary levels of drugs could replace plasma. Simple and accurate methods to estimate several anti-TB and antiretroviral drugs by high performance liquid chromatography (HPLC) in biological fluids were developed and validated. These methods were applied in pharmacokinetic studies conducted in adults and children, which addressed key issues in TB and HIV. The impact of HIV infection on anti-TB drug levels, pharmacokinetic interactions between NVP/EFV and RMP, significance of pharmacogenetics in HIV therapy were some of the aspects studied. Most of these studies were the first of its kind in Indian patients, and had important clinical implications.

Studies Completed :

(i) Pharmacokinetics of rifabutin during concomitant atazanavir / ritonavir administration in HIV-infected TB patients in India

(Collaboration: Govt. Hospital of Thoracic Medicine, Chennai)

Background: RBT is reported to be as effective against TB as RMP, and has little effect on serum concentrations of protease inhibitors. However, ritonavir (RTV), being a CYP3A4 inhibitor markedly increases serum concentrations and toxicity of RBT. Hence it becomes necessary to decrease the dose of RBT when co-administered with RTV. The dose of RBT during RTV co-administration remains a matter of debate.

Aim : (i) to study the pharmacokinetics of RBT at 150mg thrice weekly dose during concomitant atazanavir / RTV administration in HIV-infected TB patients

Methods : This observational study was conducted in 16 adult HIV-infected TB patients at the Government Hospital of Thoracic Medicine, Tambaram. These patients were being treated for TB with RBT-containing regimen and an ART regimen with RTV, the dose of RBT being 150mg thrice-weekly. Serial blood draws at pre-dosing and at 1, 2, 4, 6, 8, 12 and 24 hours after drug administration were done. Plasma RBT and RTV were estimated by HPLC.

Results & Conclusions : The demographic and clinical details of the 16 patients recruited to this study are given table 2.2. The eak concentration was below the therapeutic range (0.3 0.08µg/ml) in seven patients, while 10 patients had trough concentrations below the minimal inhibitory concentration of RBT against *M.tuberculosis* (0.06µg/ml), suggesting that RBT doses may have to be increased. The distribution of peak and trough concentrations of RBT is shown in fig. 2.4. A significant positive correlation between peak concentrations of RBT and RTV was observed (r = 72%; p = 0.002). Prospective studies in different settings are required to arrive at the proper dose of RBT to be used during RTV co-administration.

Table 2.2: Patient details

Age (years)	37.5 (29.8 – 40.0)
Males (N)	13
Body wt (kg)	54.5 (45.5 – 59.0)
Pulmonary TB (N)	8
Extrapulmonary TB (N)	8
Category I (N)	6
Category II (N)	10
Duration of ATT (months)	0.50 (0.50 – 0.50)
CD4 cell counts (cells/cu.mm)	101.5 (30.0 – 199.3)
Viral load (copies/µl)	66600 (15650 – 372250)

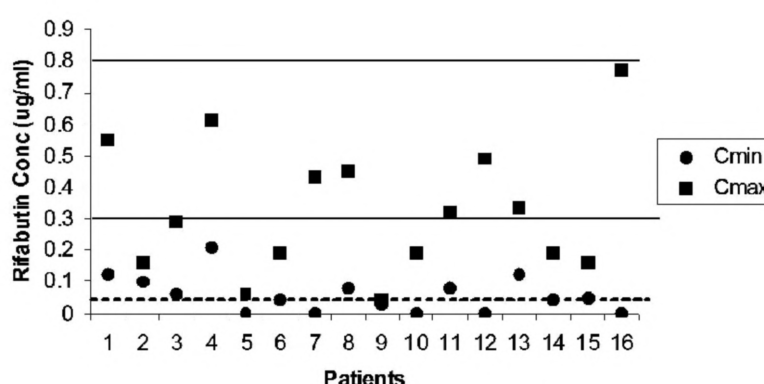

Fig. 2.4: Distribution of peak and trough concentrations of RBT

Peak concentration; Cmin: Trough concentration

Black horizontal lines denote the therapeutic range of RBT (0.3 0.8µg/ml)

Broken horizontal line denotes MIC of RBT (0.06µg/ml)

(Contact person: Dr. Geetha Ramachandran; e.mail: geethar@nirt.res.in)

Studies in progress:

(i) Pharmacokinetics of anti-TB drugs in HIV-infected children with TB

(Collaboration: ICH, Inst. Of Thoracic Medicine, KMC, Chennai and Govt. Rajaji Hospital, Madurai)

[Source of funding: ICMR Task force on Pediatric HIV]

Background: Limited information is available on the impact of HIV infection on the pharmacokinetics of anti-TB drug in children with HIV and TB. Children exhibit age-related differences in drug absorption, metabolism and clearance. HIV infection combined with intestinal dysfunction and malnutrition could cause impaired absorption of anti-TB drugs leading to reduction in blood levels of the drugs that can result in poor treatment response.

Aim: (i) to study the influence of HIV infection on the pharmacokinetics of RMP, INH and PZA in children with HIV & TB

Methods: The study population comprises of two groups of children (i) with TB alone and (ii) with HIV & TB. The required sample size is 80 in each group.

Children aged 1 to 12 years receiving treatment for TB from the RNTCP (TB treatment) centres at the Government Hospital of Thoracic Medicine, Chennai, Kilpauk Medical

College & Hospital, Chennai, Institute of Child Health, Chennai and Government Rajaji Hospital, Madurai are recruited.

The nutritional status is assessed using z scores. The pharmacokinetic study is undertaken in the respective hospitals. On the day of the study, serial blood samples at pre-dosing and at 2, 4, 6 and 8 hours of blood (2ml) is collected followed by supervised drug administration. Plasma concentrations of RMP, INH and PZA are measured by HPLC and pharmacokinetic variables calculated. TB treatment outcomes are noted from the RNTCP card.

We have recruited 84 and 64 children respectively with TB and HIV-TB. Recruitment of HIV-infected children with TB is in progress. The patient details are given in table 2.3.

Table 2.3: Demographic & clinical features of study participants

Details	TB (n = 84)	HIV & TB (n = 64)
Age (years)*	7.0 (4.0 – 10.8)	9.0 (7.0 – 11.0)
Males n (%)	40 (48)	18.0 (13.0 – 23.0)
Body wt (kg)*	42 (65.6)	17.3 (15 – 22.3)
Nutritional status*		
Height for age z score (HAZ)	- 1.2 (-2.1 - -0.3)	-3.3 (-4.3 - -2.3)
Weight for age z score (WAZ)	- 1.8 (-2.4 - -1.1)	-2.7 (-3.4 - -1.9)
Weight for Height z score (WHZ)	- 1.2 (-1.9 - -0.3)	-1.1 (-2.1 – 0.3)
Mid-arm circumference (cm)	15.0 (14.0 – 16.0)	14.0 (12.0 – 16.0)
Head circumference (cm)	49.0 (48.0 – 51.0)	48.0 (42.0 – 50.0)
Thrice weekly treatment dose mg/kg*		
RMP	9.9 (8.3 – 11.2)	10.0 (7.7 – 11.5)
INH	9.9 (8.3 – 11.2)	10.0 (7.7 – 11.5)
PZA	32.9 (27.8 – 37.2)	31.7 (17.1 – 37.5)
Duration of ATT (months)*	1.0 (0.5 – 1.0)	0.5 (0.5 – 1.0)
Regimen n (%) Category I	48 (57)	52 (81)
Category II	3 (4)	1 (2)
Category III	33 (39)	11 (17)
Type of TB n (%) Pulmonary	19 (23)	26 (41)
Extrapulmonary	63 (75)	38 (59)
Both	2 (2)	-
Rapid acetylators of INH n (%)	27 (32)	20 (31)

*Values are Median (Inter-quartile range)

(Contact person: Dr. Geetha Ramachandran; e.mail: geethar@nirt.res.in)

(ii) Comparative pharmacokinetics of RMP during daily and intermittent dosing in HIV-TB patients

(Collaboration: Govt. Hospital of Thoracic Medicine, Chennai)

Background: Intermittent RMP therapy could increase the risk of acquired RMP resistance among HIV-infected patients with TB. Sub-therapeutic plasma RMP could lead to unfavourable TB treatment outcome. A prospective pharmacokinetic study is conducted in HIV-infected patients with TB who are enrolled in a randomized controlled clinical trial in which patients receive RMP along with other medications either daily or intermittently.

Aim : (i) to study the pharmacokinetics of RMP in HIV-infected patients with TB who are receiving daily and intermittent anti-TB regimens

Methods: The study is conducted in 48 patients (24 each in the daily and intermittent dosing arms) who are getting recruited to the clinical trial at Chennai and Madurai. Eligible subjects are identified by the clinicians involved in the trial. On the day of the study, blood samples are collected at 0, 1, 2, 6, 8, 12 and 24 hrs after dosing. Plasma concentrations of RMP are estimated by HPLC according to a validated method.

So far, 39 patients have been recruited to the study. The study is in progress.

(Contact person: Dr.A.K. Hemanth Kumar; e.mail: hemanthkumarak@nirt.res.in)

(iii) Effect of plasma MFX on treatment outcome in PTB patients treated with MFX-containing anti-TB regimens

Background: Among the newer generation of fluoroquinolones, MFX has a potential to shorten TB treatment. Studies in healthy subjects have shown that RMP co-administration reduces the blood levels of MFX. But it is not clear whether the decrease would affect the treatment efficacy of MFX. Prospective studies to relate MFX blood concentrations with TB treatment outcomes are required to understand the clinical relevance of the significant pharmacokinetic interaction between MFX and RMP.

Aim: (i) to estimate plasma concentrations of MFX, RMP, INH and PZA and correlate with TB treatment outcome

Methods: This is a prospective study undertaken in PTB patients enrolled into a randomized controlled trial in which MFX is used along with other anti-TB drugs to treat TB. Blood samples at 1, 2 and 4 hrs after drug administration are collected at one month and at the end of treatment. Plasma concentrations of MFX, RMP, INH and PZA are estimated by HPLC. So far, 93 patients, 49 from Chennai and 44 from Madurai have been recruited to the study. The study is in progress.

(Contact person: Dr. Geetha Ramachandran; e.mail: geethar@nirt.res.in)

Department of Immunology

Overview: The Department of Immunology focuses on the human host immune response *in vitro* and also immunogenetics. In addition immune response in animal models is also under investigation. Not only host related parameters, but the molecular biology of the pathogen mycobacteria is also being studied.

Several serologically reactive antigens and novel T-cell antigens in culture filtrate of *M. tuberculosis* have been identified and expressed in *E .coli.* Moreover, antigens which are specifically over expressed or which exclusively appeared during oxygen stress have been identified. These dormancy associated antigens may play vital role in persistence or maintenance of persistence of TB. Whether these antigens can be used as biomarker for differential diagnosis of LTBI and PTB will be explored.

Global proteomic comparison of *M. tuberculosis* H37Rv and its isogenic *pknE* deletion mutant has revealed that PknE has a role in regulating the metabolism of *M. tuberculosis* that could enable the survival in hostile environments. The substrates of FtsY and FfH have been identified by protein interaction studies. The whole-genome sequencing of 4 clinical isolates of *M. tuberculosis*, from Tamil Nadu state has been completed.

Studies on the effect of Vitamin D3 on various immune parameters have been carried out. Vitamin D3 increased the xpression of antimicrobial peptides like Cathelicidin and Defensin via toll-like receptor (TLR) signalling and enhanced the innate immunity against PTB. It may also act as anti-inflammatory by enhancing regulatory T- cells that lead to suppressed perforin and granulysin expression at the site of infection in PTB.

Immunogenetic studies revealed that MCP-1 -2518GG genotype may be associated with protection in males and susceptibility to PTB in females, along with altered chemokine/ cytokine levels.

In TB pleurisy, increased levels of all cytokines in pleural fluid indicate TH1/TH2 mixed immune response at the site of infection. Neutrophil mediated innate immune responses in TB have been studied *in vitro:* H37Rv was more effective in activating neutrophils and in turn stimulating monocytes and T-cells. In comparison, vaccine strains were less effective in modulating neutrophil functions. The increased expression of various signaling receptors along with the activation markers on H37Rv infected neutrophils indicated its strong capacity to activate and initialize the immune response.

Studies completed:

(I) Effect of vitamin D_3 on Cathelicidin, Defensin-1 and Toll like receptor gene expression in the neutrophils of PTB patients

Background: Neutrophils are essential components of the human innate immune system and associated with the first line defense mechanism against invading microorganisms. Vitamin $D_{3,}$ a potential immunomodulator, is known to influence innate and adaptive immunity. In the present study, the effect of vitamin D_3 on the innate immune functions of neutrophils in PTB is explored at the molecular level using RT-PCR.

Aim: (i) to find out vitamin D_3 effect on cathelicidin, defensin-1a and TLR gene expression in the neutrophils of PTB patients

Methods: The study was carried out in 20 PTB patients and 20 healthy control subjects. Neutrophils isolated from heparinized blood by Ficoll-Hypaque gradient centrifugation followed by sedimentation in 3% Dextran. Neutrophils were cultured for 18 hrs with live

M. tuberculosis and its culture filtrate antigen (CFA) in the presence and absence of vitamin D_3. The total RNA extracted was used for complementary DNA (cDNA) synthesis. Relative quantification of the target genes cathelicidin, defensin-1á, vitamin D receptor (VDR), TLR-2,4,8,9, TIRAP genes and house keeping gene ß -actin was carried out using RT-PCR with TaqMan assay primers and probes.

Results: Vitamin D_3 significantly increased the relative fold expression of cationic anti-microbial peptide (CAMP), Defensin- a 3, TLR-2, TLR-9 and TIRAP genes as compared to ethanol treated control neutrophils in both the study groups ($p<0.05$). Vitamin D_3 treatment significantly up regulated CAMP expression in CFA stimulated and *M.tb* infected neutrophils in HCs (p=0.0007 and p=0.0079) and PTB patients (p=0.0022 and p=0.0069) (Fig.2.5). In *M.tb* stimulation, TLR9 expression was significantly increased as compared to unstimulated cells ($p<0.05$) in both the study groups. There was no significant effect of vitamin D_3 on TLR4 and TLR 8 mRNA expression in controls and PTB patients.

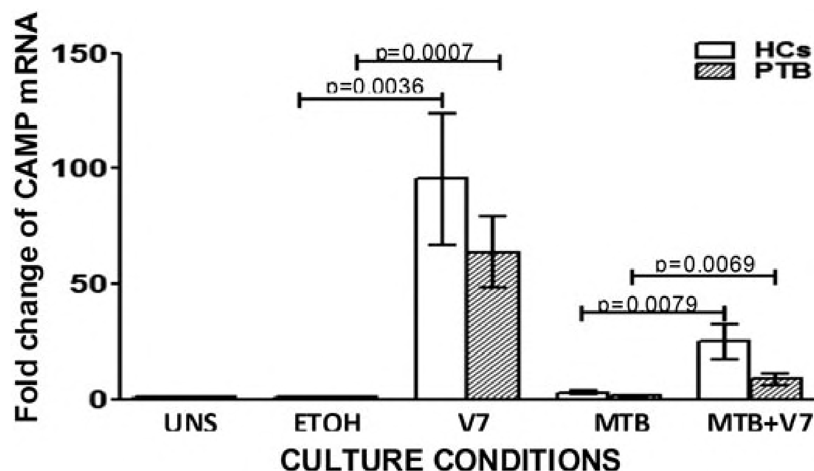

Fig. 2.5: Effect of vitamin D_3 on the relative expression of CAMP mRNA

Uns: Unstimulated cells; ETOH: Ethanol control; V7: Vitamin D_3 $1x10^{-7}$M concentration;
Mtb: *M. tuberculosis*; HCs: Healthy controls; PTB: Pulmonary tuberculosis patients

Conclusion and Implication : The study results suggested that vitamin D_3 may increase the TLR expression and lead to increased expression of antimicrobial peptides via TLR signalling and enhance the innate immunity against PTB.

(Contact person: Dr. P. Selvaraj, e.mail: selvarajp@nirt.res.in)

(ii) Effect of vitamin D_3 on intracellular expression of perforin, granulysin and regulatory T-cells in PTB

Background: Protective immunity in TB is dependent on the co-ordinated release of cytolytic effector molecules from effector T-cells and the subsequent granule-associated killing of infected target cells. Vitamin D_3 is a potent modulator of macrophage and lymphocyte functions and enhances the exocytosis of cytolytic granules like perforin and granzymes and antimicrobial molecules such as granulysin from cytotoxic T-lymphocytes. T-regulatory (Treg) cells have been shown to suppress antimicrobial immune responses against intracellular pathogens and protect the host by preventing collateral damage from excessive inflammation. The present study was aimed to understand the effect of vitamin D_3 on intracellular expression of various cytolytic molecules and regulatory T-cells in PTB.

Aim: (i) to find out the effect of vitamin D_3 on intracellular expression of perforin, granulysin and regulatory T-cells in PTB

Methods: The study was carried out in 20 PTB patients and 20 healthy control subjects. Peripheral blood mononuclear cells were cultured for 72 hrs with live *M.tuberculosis* and its CFA in the presence and absence of vitamin D_3. After 72 hrs, the cells were processed for immunostaining of CD4, CD8, CD25 and CD56 cell surface markers and intracellular perforin, granulysin and Foxp3$^+$ for regulatory T-cells by using specific monoclonal antibodies and analyzed in flow cytometry.

Results: Vitamin D_3 significantly suppressed the expression of perforin (Fig. 2.6) and granulysin (Fig. 2.7) of *M.tb* and CFA stimulated total cells, CD8 and CD56 positive cell subsets ($p<0.05$). A significantly higher expression of Tregs was found in vitamin D_3 treated cells compared to ethanol treated control cells ($p<0.05$) in healthy controls (HCs) and PTB patients. Vitamin D_3 significantly enhanced the FoxP3 expression in the regulatory T-cells positive for CD4 and CD25 as compared to live M.tb and CFA treated cells in both the study groups ($p<0.05$). PTB patients showed higher Treg positive cells compared to healthy controls.

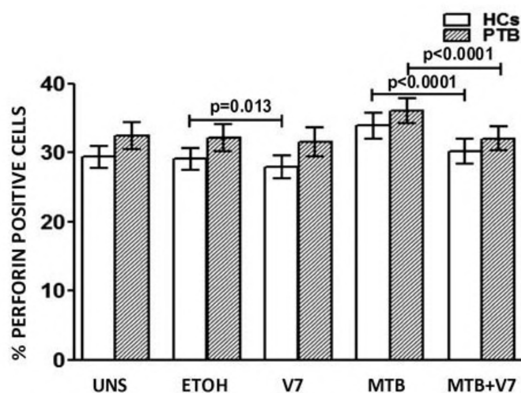

Fig. 2.6: Effect of vitamin D_3 on intracellular expression of Perforin

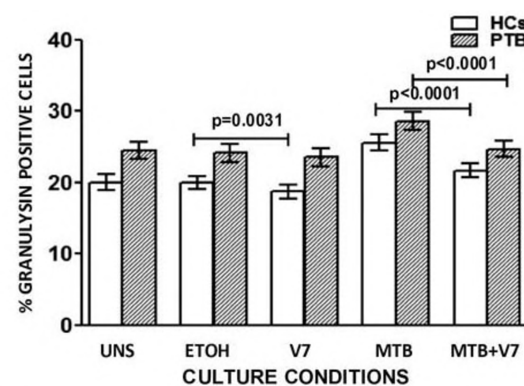

Fig. 2.7: Effect of vitamin D_3 on intracellular expression of Granulysin
Uns: Unstimulated cells; ETOH: Ethanol control; V7: Vitamin D_3 $1X10^{-7}$M Concentration; Mtb: *M. tuberculosis*; HCs: Healthy controls; PTB: Pulmonary tuberculosis patients.

Conclusions and Implications: Vitamin D_3 may act as anti-inflammatory by modulating T-cell response in adaptive immunity by enhancing regulatory T- cells that may lead to suppressed perforin and granulysin expression at the site of infection in PTB.

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(iii) CCL2 (MCP-1), CCL3 (MIP-1) and CCL4 (MIP-1) gene polymorphisms in PTB patients of south India

Background: Host genetic factors were suggested to play an important role in genetic makeup and to determine the inter-individual variation. Chemokines are small, secretory chemotactic cytokines which mediate constitutive recruitment of leukocytes from blood to the site of infection. Earlier studies revealed that genetic variations in chemokine gene are associated with differences in disease susceptibility and clinical manifestations.

Aim: (i) to find out whether various CC chemokine gene polymorphisms as well the haplotypes of these genes are associated with susceptibility or resistance to PTB in south Indian population

Methods : Polymorphisms of the various CC chemokine genes, MCP-1 (-2518A/G, 903C/T), MIP-1 (-2021C/T, +740A/G) and MIP-1 (-5725A/C) were studied in 295 HCs and 303 PTB patients using polymerase chain reaction based restriction fragment length polymorphism (PCR-RFLP).

Results: The allele and genotype frequencies were not significantly different between the study subjects. However, a significantly decreased frequency of GG genotype was observed in male PTB patients as compared to male HCs (p value=0.015, OR 0.43 95% CI (0.21-0.86)) and an opposite trend of increased frequency of GG genotype was observed among female PTB patients as compared to female HCs (p value=0.049, OR 2.28 95% CI (1.00-5.27)). Moreover, we also observed significant differences in the haplotype frequencies of these chemokine genes between HCs and PTB patients.

Conclusion and implication: The results suggest that MCP-1 -2518GG genotype may be associated with protection in males and susceptibility to PTB in females. This genotype in combination with other functional polymorphisms may be associated with altered chemokine / cytokine levels and result in the development of TB or protection against disease progression.

(Contact person: Dr. P. Selvaraj, e.mail: selvarajp@nirt.res.in)

(iv) A mixed immune response at the site of infection in tuberculous pleurisy

Background: The entry of mycobacterial antigens into the pleural space triggers local, delayed type hypersensitivity reaction leading to the recruitment of $CD4^+$ cells to the site of infection in tuberculous pleurisy (TP). In TB, the inflammatory response is generally T helper (Th) 1 driven which provides protection against mycobacterial infections. Recently discovered Th17 cells are also important in inducing optimal Th1 response but its role in TP remains unclear.

Aims: (i) to compare the levels of Th1, Th2 and Th17 cytokines in plasma (BL) and pleural fluid (PF) of TB and non-TB (NTB) patients and explore the possibility of using them as potential additional markers for the diagnosis of the disease

Methods : Blood and PF samples were collected from 44 patients, of them 28 had exudative pleural effusions with lymphocytic predominance (TB) and 16 had non-TB etiology and formed control group (NTB). Cytokines were estimated using commercially available Bioplex multiplex cytokine assay system (Biorad, Hercules, CA) following the protocols specified by the manufacturers.

Results: The levels of Th1 cytokines (IFN-gamma, TNF-alpha, IL-2, IL-12 and GM-CSF) (Fig. 2.8), Th2 (IL-4) (Fig. 2.9) and Th17 (IL-6, IL-1beta and IL-17) (Fig. 2.10) were significantly higher in PF than BL in both TB and NTB groups. On the contrary, the levels of other Th2 cytokines (IL-10 and IL-13) (Fig. 2.9) were significantly higher in PF compared to BL only in TB group. When compared between the groups, significantly higher levels of IL-6, IL-10, IL-12 and IL-13 in BL and IL-10 in PF were observed in NTB.

Conclusion: In TB pleurisy, increased levels of all cytokines in PF indicate mixed immune response at the site of infection. Moreover, the differential Th2 cytokines (IL-10 and IL-13) can be explored for definite diagnosis of extra PTB like TP.

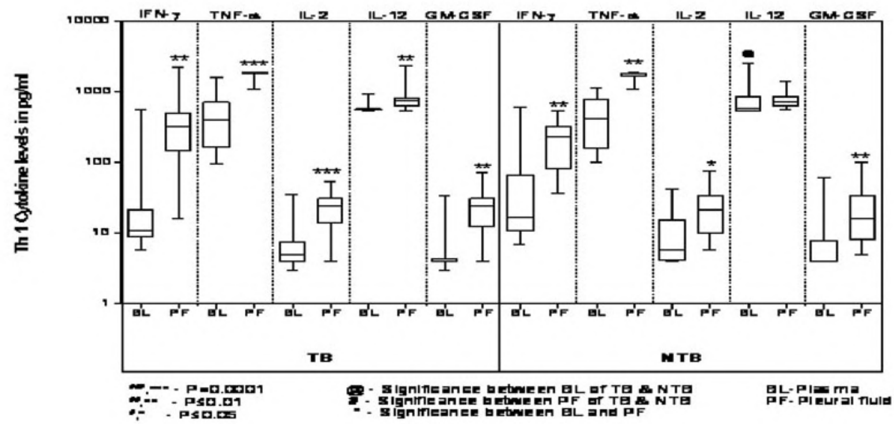

Fig 2.8: Levels of Th1 cytokines in plasma (BL) and pleural fluid (PF) of tuberculous (TB) and non-tuberculous pleuritis (NTB) patients

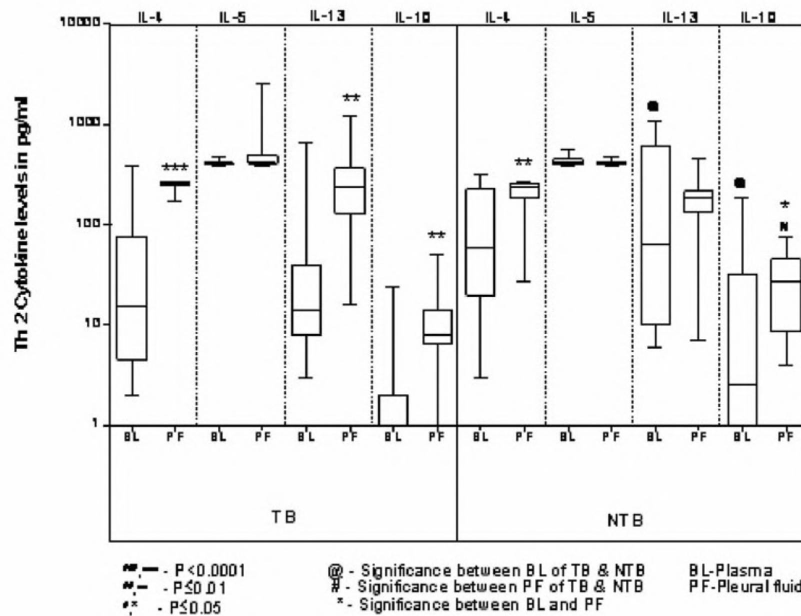

Fig 2.9: Levels of Th2 cytokines in plasma (BL) and pleural fluid (PF) of tuberculous (TB) and non-tuberculous pleuritis (NTB) patients

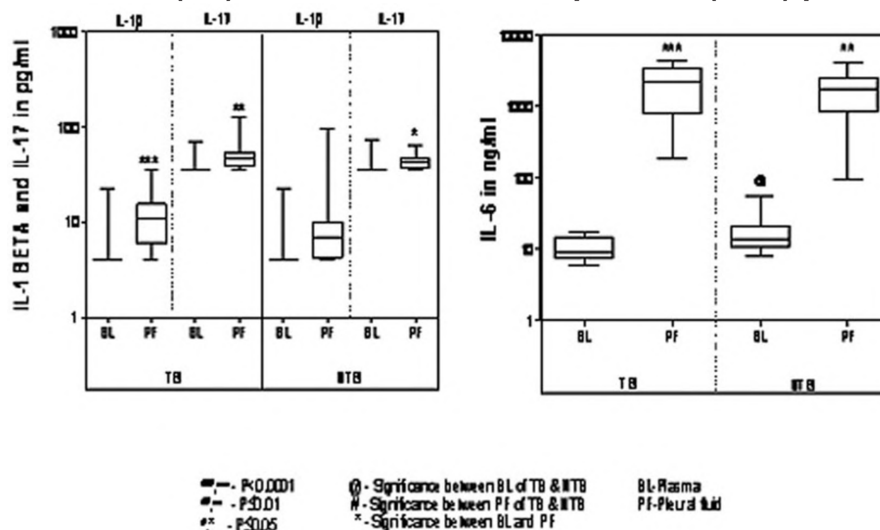

Fig 2.10: Levels of Th17 cytokines in plasma (BL) and pleural fluid (PF) of tuberculous (TB) and non-tuberculous pleuritis (NTB) patients

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Studies in progress:

(I) Identification of serologically reactive antigens in culture filtrate of *M. tuberculosis*

Background: Detection of *M. tuberculosis* (*M. tb*)-speci? c human antibodies has been an important diagnostic aid in the diagnosis of TB. Proteins secreted into the extracellular environment by *M. tb* are recognized by the immune system in the infected host, constituting an important source of antigens that induce immune responses with diagnostic value.

Aim: (i) to identify the serologically reactive fractions in CFA of *M. tb*

Methods: **C**FA of *M. tb* was subjected to preparative 2-dimensional electrophoresis which separated CFA into 600 fractions. To identify serologically reactive fractions, each fraction was tested with pooled sera of 50 TB patients and 50 healthy control subjects (HCS) by indirect ELISA. Fold difference of each fraction was calculated by OD of TB sera / OD of control sera. Identified individual fractions were assessed for their serodiagnostic potential with 110 sera from HCS and 88 sera from PTB. Cut off value for each fraction was ascertained by mean +2SD.

Results: Out of 600 fractions tested, 16 fractions showed = threefold reactivity in TB sera than control sera (Table 2.4). On assessing 16 individual fractions, sensitivity ranges from 10% to 34% in PTB, with the specificity ranging from 93% to 100% in HCS. While combining fractions, 8 fraction combinations (6_14, 18_02, 14_04, 18_03, 8_11, 13_05, 14_03 and 2_28) increased sensitivity to 87.5% in PTB with the specificity of 88% in HCS (Table 2.5).

Table 2.4: IEF_WGE fractions and their fold difference

No. of IEF_WGE fractions	Fold Difference
16	e” 3
94	e” 2
392	< 2

Table 2.5: Sensitivity and specificity of individual and combination of fractions

IEF_WGE Fractions	% Specificity	% Sensitivity		
		HHC	PTB	HIV TB
2_25	99.09	10.91	10.23	4.55
2_28	99.09	15.45	11.36	12.5
2_29	96.36	7.27	12.5	1.14
5_10	98.18	8.18	13.64	5.68
6_14	99.09	7.27	34.09	11.36
7_14	95.45	8.18	12.5	7.95
8_07	93.64	15.45	28.41	22.73
8_09	98.18	11.82	17.05	19.32
8_11	99.09	13.64	23.86	20.45
13_02	96.36	17.27	28.41	17.05
13_05	98.18	4.55	18.18	4.55
14_03	100	0.91	13.64	4.55
14_04	97.27	2.73	29.55	13.64
15_28	96.36	2.73	29.55	28.41
18_02	98.18	13.64	27.27	14.77
18_03	98.18	10.91	31.82	13.64
2_28 + 6_14 + 8_11 +13_05 +14_03 + 14_04 +18_02 + 18_03	87.5	41.8	88	63.6

Conclusion: These eight fractions were identified as serologically reactive fractions and the antigens present in these fractions would be the promising candidates for serodiagnosis of TB.
(Contact person: Dr. Alamelu Raja email: alamelur@nirt.res.in)

(ii) Dormancy associated antigens of *M. tuberculosis*

Background: Analysis of the mycobacterial antigens associated with the slowly replicating, post-logarithmic phase growth of *M. tuberculosis*, the so-called "dormant" phase, is our interest. Three different strains of *M. tuberculosis* were used in this study: the laboratory strain H37Rv and 2 of the clinical strains, most prevalent in south India, S7 and S10. Clinical strains were used since no literature reported dormancy associated antigens from clinical strains.

Aim: (i) to identify and proteomically characterize dormancy associated antigens of *M. tuberculosis*

Methods: H37Rv, S7 and S10 strains were grown aerobically (MB7H9 media, 300rpm, 37°C) and anaerobically (0.5 ratio of headspace air volume to liquid volume, MB7H9, 120 rpm, 37°C). Cytosolic antigens were prepared and separated by 2-D electrophoresis (Bio-Rad Laboratories, USA) and differentially expressed protein spots were characterized by mass spectrometry (AB Sciex, Massachusetts, USA).

Results: Oxygen depletion was set up by Waynes's model and discolorization of methylene blue indicates oxygen depletion from the media**.** In order to investigate proteins expressed by hypoxia cultures, cell lysate proteins of *M. tuberculosis*, S7 and S10 were analyzed by 2-D electrophoresis and protein spot patterns were compared with those of aerated cultures (Fig. 2.11). The protein spots differing between the aerobic and anaerobic cultures were excised from the gel and identified by liquid chromatography tandem mass spectrometry (LC/MS/MS). Spots that have increased intensity, compared to aerobic cultures, are considered as over expressed proteins under anaerobic conditions. Rv2445, Rv0440, RvRv3133c, Rv1908 are characterized as over expressed spots. Few spots, Rv0251, Rv2031, Rv2185c, Rv3592, appeared only on oxygen depletion and considered as specific proteins for oxygen depletion.

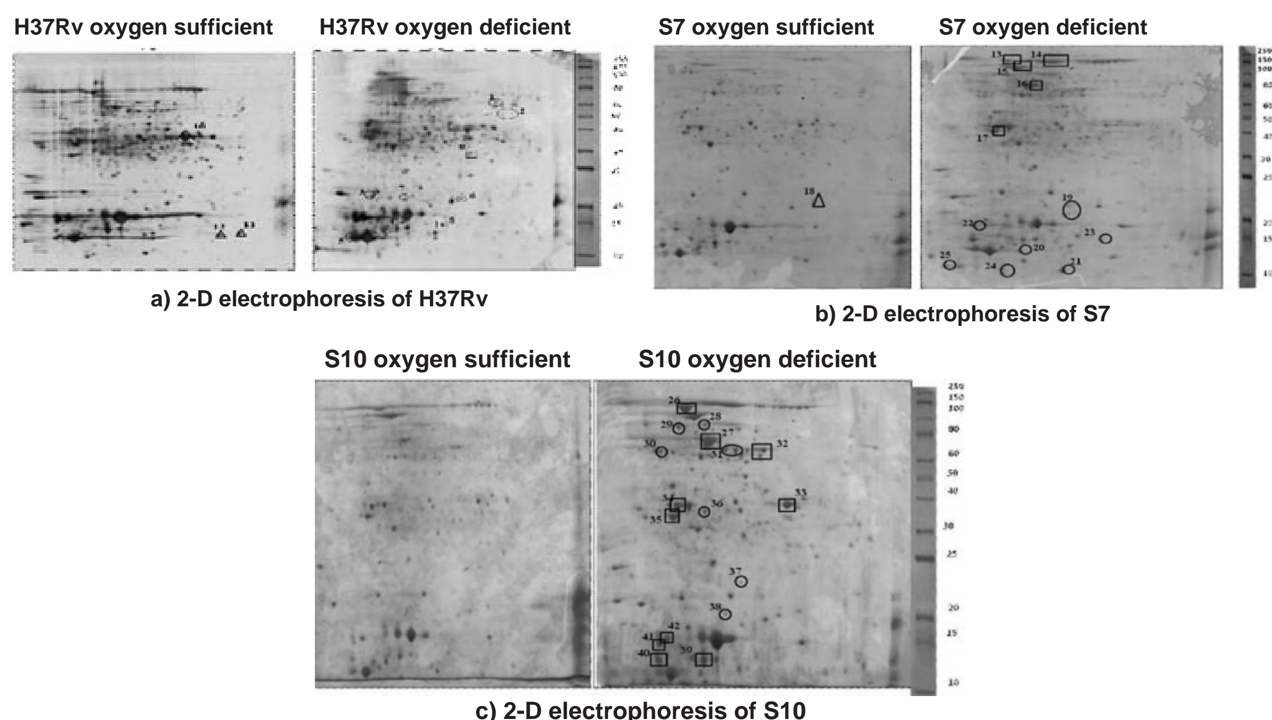

Fig. 2.11: Two dimensional electrophoresis of cytosolic proteins of *M. tuberculosis*
Circled- New spots, Boxed over-expressed spots, Triangle Under expressed spots under oxygen depletion

Conclusion: A few proteins were expressed only under oxygen depleted conditions of H37Rv and characterized as hypothetical proteins (Rv2953, Rv2185c, Rv3466 and Rv0854) and are found to be conserved among mycobacterial species. In our observation, Rv2031c appeared in H37Rv and S7 and over expressed in S10, during oxygen tension. Mycobacterial chaperon protein GroEL 2 (Rv0440) was over expressed in both of clinical isolates used but not in H37Rv when oxygen is depleted from the culture media. Rv3396c also was found to be expressed only during oxygen stress in S10 clinical strains of *M. tuberculosis*. It is predicted to be Guanosine Monophosphate Synthase is a key enzyme in the purine biosynthetic pathway.

Compared to other components, stress-associated proteins have received little attention as antigens for diagnosis and vaccine development against TB. Thus antigens which are specifically over expressed or appeared during oxygen stress may play some vital role in persistence or maintenance of persistence of TB.

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(iii) Immunoproteomically idenditified *M. tb* antigens for differential diagnosis of LTBI and active TB disease

Background: An estimated one-third of the world's population who harbor *M. tb* remains asymptomatic and termed latently infected (LTBI). Early diagnosis and treatment of LTBI individuals are crucial in effective TB control programmes. The existing TST and IFN- release assays (IGRAs) are inefficient diagnostic tools for LTBI detection. Earlier we had carried out the separation of culture filtrate proteins (CFP) of *in vitro* grown *M. tb* by two dimensional-liquid phase electrophoresis and tested for the ability to stimulate T-cells in human models of TB. Of those, 10 fractions were exclusively recognized only by the healthy household contacts (HHC) and not by TB patients. A total of 16 proteins were identified from the 10 contact specific (CS) fractions by using LC-MS/MS method.

Aim: (i) to analyze t-cell mediated *in vitro* cytokine response to differentiate LTBI and active TB, by using 16 antigens. The aim will be fulfilled by; cloning, over expression and purification of all 16 antigens; comparison of IFN- levels in response to these antigens by using whole blood from the study subjects

Methods: pET/pRSET vectors were used to construct desired recombinant plasmids. Upon induction with 1mM IPTG, all the recombinant proteins were over expressed by using BL21 (DE3)/ BL21 (DE3) pLysS *E. coli* system.

Results: Table 2.6 indicates the list of antigens present in the 10 fractions, which were exclusively recognized only by HHC and not by TB patients. All the over expressed recombinant proteins were purified by affinity column chromatography (Fig. 2.12). Some of the proteins (ESAT-6, CFP-10, Ag85B and GroES) were kind gift from Colorado State University, USA.

Conclusion: All the purified antigens will be further used to characterize the T-cell mediated *in vitro* cytokine response (IFN-) to differentiate LTBI and active TB, by using whole blood from the study subjects.

Table 2.6: List of proteins present in 10 CS fractions

Protein Fraction	Protein name	Gene number	Sub cellular location
7_28	GroES	Rv3418c	Cytoplasm
8_29	AcpM	Rv2244	Cytoplasm
8_29	ESAT-6	Rv3875	Secreted
8_29	TB8.4	Rv1174c	NA
9_24	CFP-10	Rv3874	Secreted
9_24	Rv3716c	Rv3716c	NA
9_24	TrxC	Rv3914	NA
9_26	HspX	Rv2031	Secreted
10_11	DnaK	Rv0350	NA
10_11	Phos1	Rv0934	Cell membrane
10_11	Pst2	Rv0932c	Cell membrane
11_24	FbpB	Rv1886c	Secreted
11_24	Rv2626c	Rv2626c	NA
12_21	ADK	Rv0733	cytoplasm
12_21	KatG	Rv1908c	NA
12_23	GroEL	Rv0440	cytoplasm

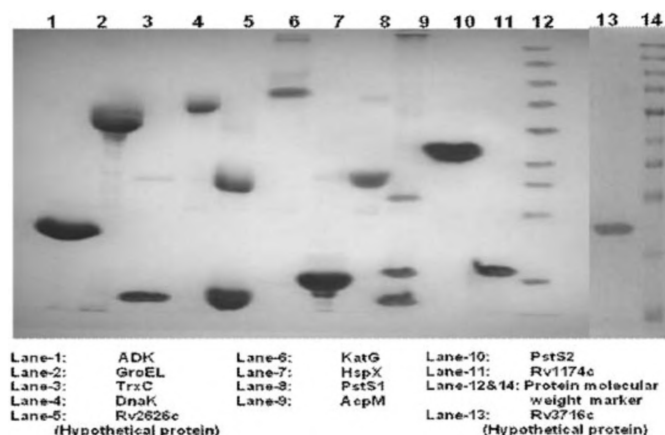

Fig. 2.12: Purified recombinant proteins in 12.5% SDS-PAGE gel

(Contact person: Dr. Alamelu Raja, email: alamelur@nirt.res.in)

(iv) Immunological characterization of novel T- cell antigens of *M. tuberculosis*

Background: PpiA (Rv0009), Rv2204c (hypothetical protein) and mmsA (Rv0753c) are the novel T-cell antigens, producing higher levels of IFN- in latently infected individuals (healthy household contacts) than active TB patients which were identified in our earlier studies. Bioinformatics analysis also predicted that these proteins had a wider class I and class II HLA binding and also more population coverage compared with the well-reported T-cell antigens (ESAT-6 and CFP-10). These results provoked us to choose these proteins for further immunological characterization in terms of their usage in differentiating LTB and active TB. PpiA protein purification has been reported in 2011-2012 report.

Aim: (i) to analyze the T-cell mediated *in vitro* cytokine response (particularly IFN-) to differentiate LTBI and active TB disease

Methods : Rv2204c, Rv0753c genes were amplified by using H37Rv genomic DNA of *M. tb* and cloned in pRSET-A vector. The recombinant protein was over expressed and purified in *E. coli* expression system [BL21 (DE3)]. Purification was done by affinity column

chromatography (Ni-NTA). Blood was drawn from 15 HHC subjects (subjects were quantiferon positive and known to be exposed to active TB patients) and 19 active TB subjects (QFT positive, smear positive) and diluted (1 in 10) with RPMI. The diluted blood was stimulated against purified recombinant proteins with final concentration 5 g/ml and cultured for 6 days at 37°C. Cell culture supernatant was harvested on 6th day and IFN- levels were measured by ELISA.

Results : Rv2204c (13KDa) and Rv0753c (55KDa) recombinant proteins were purified by affinity column chromatography (Fig.2.13). Endotoxin contamination of these recombinant proteins ranged from 1 - 1.5ng/mg, which was estimated by Limulus amebocyte lysate assay. All the three antigens induced significantly higher IFN- production in HHC compared to active TB subjects (Fig. 2.14).

Conclusion : This preliminary study shows, these three antigens are differentiating LTBI from active TB disease in terms of IFN- levels. Number of study subjects will be increased in this direction which may aid to confirm whether these antigens can be used as biomarker for differential diagnosis of LTBI and PTB.

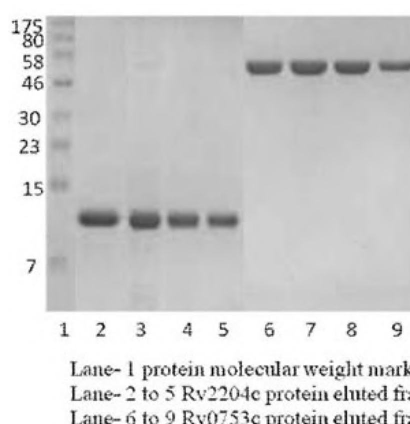

Fig. 2.13: Eluted protein fractions of Rv2204c and Rv0753c in SDS PAGE gel

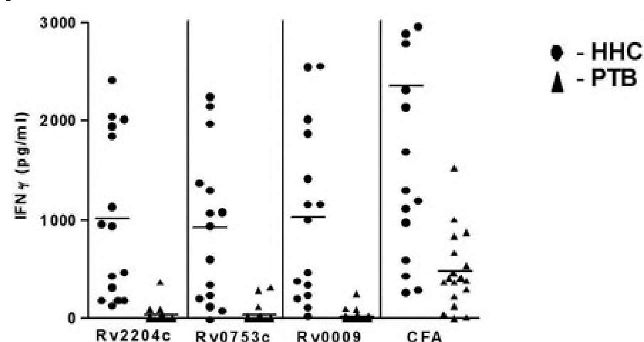

Fig. 2.14: IFN- response against 3 antigens

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(v) Structural characterization of three essential genes from *M. tuberculosis*

Background: Search for new drug targets for treating TB, is essential since XDR and MDR-TB are more prevalent. Previously in our laboratory, proteomic characterization of low abundant proteins in the CFPs of *M. tb* (H37Rv strain) was carried out. The experiments (*in vitro* and *in silico*) showed that three proteins (Rv1177, Rv2675c and Rv3503c) had no human homologues and hence have potential to be tested as drug target against TB. The current study is initiated with the expression of these three antigens of *M. tuberculosis* to target for drug discovery.

Aim: (I) to clone, overexpress and purify three proteins coded by genes (Rv1177, Rv2675c and Rv3503c) from *M. tuberculosis*, study the structure of these proteins and check whether these proteins can be used as drug targets for TB

Methods: All the three genes were PCR amplified using H37RV strain of *M. tuberculosis* as template. Cloning of each individual gene is performed using pET TOPO directional cloning vector. The cloned genes are transformed into the expression strain (BL21 star DE3) and the proteins are over-expressed by Isopropyl -D-1-thiogalactopyranoside (IPTG) induction. The proteins are then purified by Ni-NTA column (affinity chromatography).

Results: Amplification of all the three genes was done by PCR in appropriate conditions of melting temperature (Fig. 2.15). The amplified and PCR purified genes of interest were cloned into pET TOPO directional cloning vector and the recombinant vectors were transformed into TOP10 cells. A colony PCR was performed to confirm the presence of the genes of interest in the colonies (Fig. 2.16) and the recombinant vectors were transformed into the expression strain (BL21 star DE3).

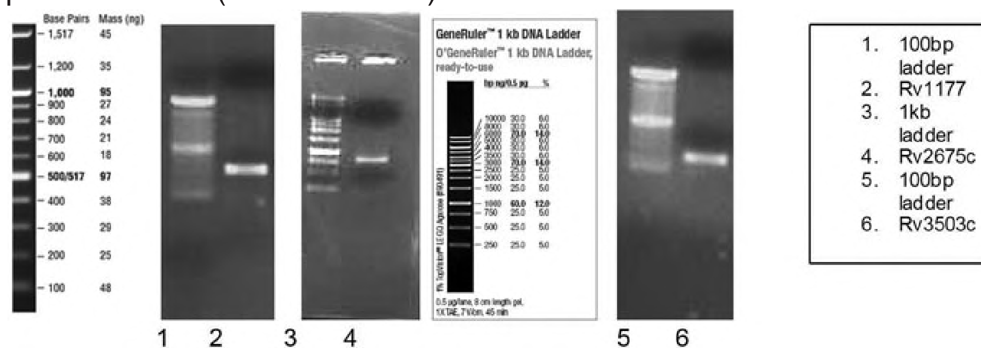

Fig. 2.15: PCR amplification of Rv1177 (327bp), Rv2675c (753bp) and Rv3503c (192bp)

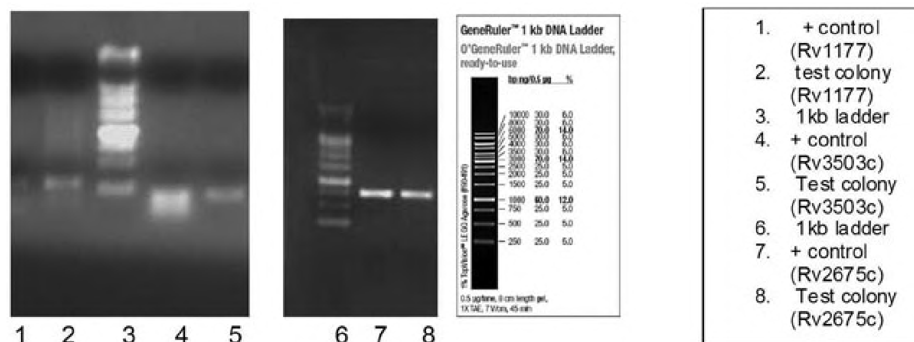

Fig. 2.16: Colony PCR of the recombinant genes after cloning into pET TOPO directional cloning vector

Conclusion: The cloned genes are confirmed using colony PCR and vector PCR. Expression of the individual proteins will be carried out in appropriate conditions after standardizing the temperature for expression and studying the time kinetics.

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(vi) Knock out mutant of *lpqS* in *M. tuberculosis* is attenuated in guinea pig model of infection

Background: Lipoproteins constitute about 2,5 % of the total *M. tuberculosis* proteome. Deletion of the lipoprotein gene *lpqS* (Rv0847) from the genome of *M. tuberculosis* results in defective *in vitro* growth of the mutant. The mutant is also attenuated for growth in macrophage model of infection. Complementation of the mutant fails to restore the wild type characteristics.

Aims: The main objective of the study is:

(i) to study the intracellular viability of the mutant in guineapig model of infection and

(ii) to analyze whether Rv0847(*lpqS*)-Rv0850 are expressed as an operon

Methods: Guinea pig infection with *M. tuberculosis*

Pathogen free 200300g female outbred Dunkin Hartley guinea pigs were housed in stainless steel cages and were provided with ad libitum food and water in a BSLIII facility (National JALMA Institute of Leprosy and Other Mycobacterial Diseases, Agra, India). All the experimental protocols were reviewed and approved by the animal ethics committee of the institute. Guinea pigs (n = 6) were infected with 20-50 bacilli of each of virulent *M. tuberculosis* H_{37}Rv, △*lpqS* mutant and the complemented strain C△*lpqS* *via* the respiratory route in an aerosol chamber (Inhalation Exposure System, Glasscol Inc., IN, USA). Four weeks after infection animals were euthanized by i.p. injection of Thiopentone sodium (100 mg/kg body weight) (Neon Laboratories Ltd., India). After aseptically dissecting the animals, lung, liver and spleen were examined for gross pathological changes and scored using the Mitchison scoring system. Specific portions of lungs and spleen were then weighed and homogenized separately in 5 ml saline in a Teflon glass homogenizer. Appropriate dilutions of the homogenates were inoculated onto 7H11 agar plates in duplicates and incubated at 37°C in a CO_2 incubator for 3 4 weeks. The number of colonies were counted and expressed as $\log_{10}$CFU/g of tissue.

RT-PCR Analysis : Isolation of total RNA from *M. tuberculosis*

Cells of a 50 ml *M. tuberculosis* culture was resuspended in 4 ml of Trizol (Invitrogen) and vortexed to make a uniform suspension. They are then aliquoted as 1 ml suspension into vials containing 0.1mm zirconium beads. Cells were then disrupted using mini bead beater for 10 rounds at a maximum speed for 30 seconds. The lysate was then centrifuged at 10,000 rpm for 20 minutes at 4°C. Supernatant was then transferred to another fresh autoclaved vial and the total RNA purified using an RNeasy purification kit (Qiagen). 10   g of RNA was then made upto 50   l with DNaseI buffer and added 1   l of RNase inhibitor and 2   l DNaseI and incubated at 37°C for 45 minutes. The sample was then treated with 0.5   l of 0.5M EDTA and incubated at 75°C for 10 minutes. PCR was carried out with gene specific primers to confirm the absence of DNA contamination. A small aliquot (3   l) of RNA was then denatured at 65°C for 15 minutes in a new PCR tube and quantified using nanodrop. cDNA synthesis was then performed using Quantitech Reverse transcriptase kit (Qiagen). Reverse transcriptase negative (RT-ve) reactions lacking reverse transcriptase enzyme was also carried out simultatnously to confirm that the RNA extracted is free of DNA contamination. RT-PCR reactions were then carried out for the intergenic regions of the *lpqS* gene cluster (*lpqS*- Rv0850). Primer pairs used for RT-PCR experiments are given in table 2.7

Table 2.7: Primer pairs used for RT-PCR

Intergenic Region no.	Primer pair	Intergenic region amplified	Amplicon size
1	*lpqS* F1 5'-GTTCTGCCTGGCTCGTCGCT-3' *cysK2*R1 5'-GTTGGAGGTGATGCTTGTTG-3'	*lpqS-cysK2*	183bps
2	*lpqS* F2 5'-TTCGCACCGGTCAAGACCTG-3' *cysK2*R2 5'-AGTGACTTGATATCCCTCCG-3'	*lpqS-cysK2*	300bps
3	*cysK2*F1 5'-ACACGCAGCACCACGGTGAT-3' Rv0849R1 5'- AAATTGCGCACGCCCATCAC-3'	*cysK2*-Rv0849	220bps
4	*cysK2*F2 5'- CATCTACAACGACGCGTACT-3' Rv0849R1 5'- AAATTGCGCACGCCCATCAC-3'	*cysK2*-Rv0849	234bps
5	Rv0849F1 5'-CGTCGGTTGGACACATTCAC-3' Rv0850R1 5'-TCCTGATTGAATACCGCACG-3'	Rv0849-Rv0850	240bps
6	*cysK2*F1 5'-ACACGCAGCACCACGGTGAT-3' Rv0850R1 5'-TCCTGATTGAATACCGCACG-3'	*cysK2*-Rv0850	1473bps
7	*lpqS* F1 5'-GTTCTGCCTGGCTCGTCGCT-3' Rv0849R1 5'-AAATTGCGCACGCCCATCAC-3'	*lpqS*-Rv0849	1527bps

△*lpqS* mutant is attenuated in guinea pig model of infection

To determine whether LpqS is required for adaptive survival upon entering a mammalian host, guinea pigs were infected with *M. tuberculosis* H_{37}Rv, MTB△*lpqS* and the complemented strain C△*lpqS via* the respiratory route in an aerosol chamber calibrated to deliver approximately 20-50 bacilli to the lung. Six animals were included in each group. The infecting bacilli were previously grown to early exponential phase (OD_{600}-0.5) in enriched 7H9-OADC-Tween20 medium. Animals were sacrificed after four weeks of infection. On comparing the number and size of the gross lesions in lung, liver and spleen it was observed that the organs of animals infected with H_{37}Rv exhibited extensive involvement and were characterized by the presence of numerous large tubercles and areas of necrosis compared to organs of guinea pigs infected with the MTB△*lpqS*. Organs of guinea pigs infected with mutant exhibited minimal involvement with one or two large tubercles or moderate number of just visible tubercles. Complementation of the mutant resulted in only partial restoration of the wild type virulence trait. The △*lpqS* mutant exhibited significantly reduced size and number of lesions in all the organs evaluated when compared with wild type H_{37}Rv infected animals. Bacterial load in the lung and spleen of the infected animals were counted (Figs. 2.17 & 2.18). Significant reduction of about 2 log CFU in the bacillary load of lung and spleen was observed with the mutant infected guinea pigs compared with the guinea pigs infected with *M. tuberculosis* H_{37}Rv. Complementation of the mutant only partially restored the wild type virulence trait.

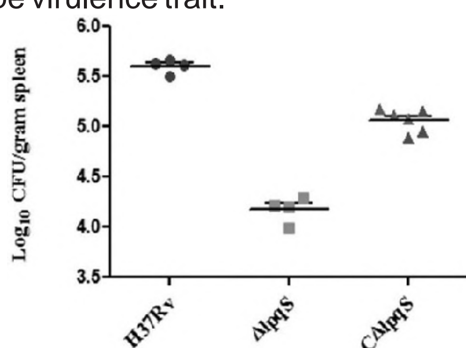

Fig. 2.17: Lung CFU obtained after infection of guineapigs with H37Rv, △lpqS mutant and complement

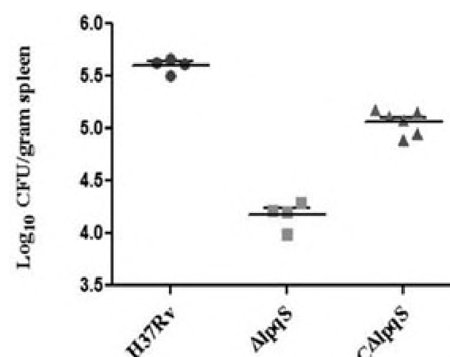

Fig. 2.18: Spleen CFU obtained after infection of guineapigs with H37Rv, △lpqS mutant and complement

RT-PCR analysis

Unsuccessful attempts to achieve full complementation of the mutant with a single copy of the *lpqS* gene cloned under the hsp60 promoter prompted us to look for the possibility that *lpqS* forms an operon with the genes downstream. RT-PCR analysis was used to identify whether *lpqS* and the three genes downstream are cotranscribed using primers amplifying intergenic regions of the gene cluster. cDNAs were synthesized from RNA of *M. tuberculosis* using Quantitect Reverse transcriptase kit from Qiagen. Transcripts were detected for all intergenic junctions tested (Fig. 2.19). Negative control experiments were performed by excluding the reverse transcriptase enzyme during RT-PCR reactions, which also served as means of confirming the absence of DNA contamination in RNA samples.

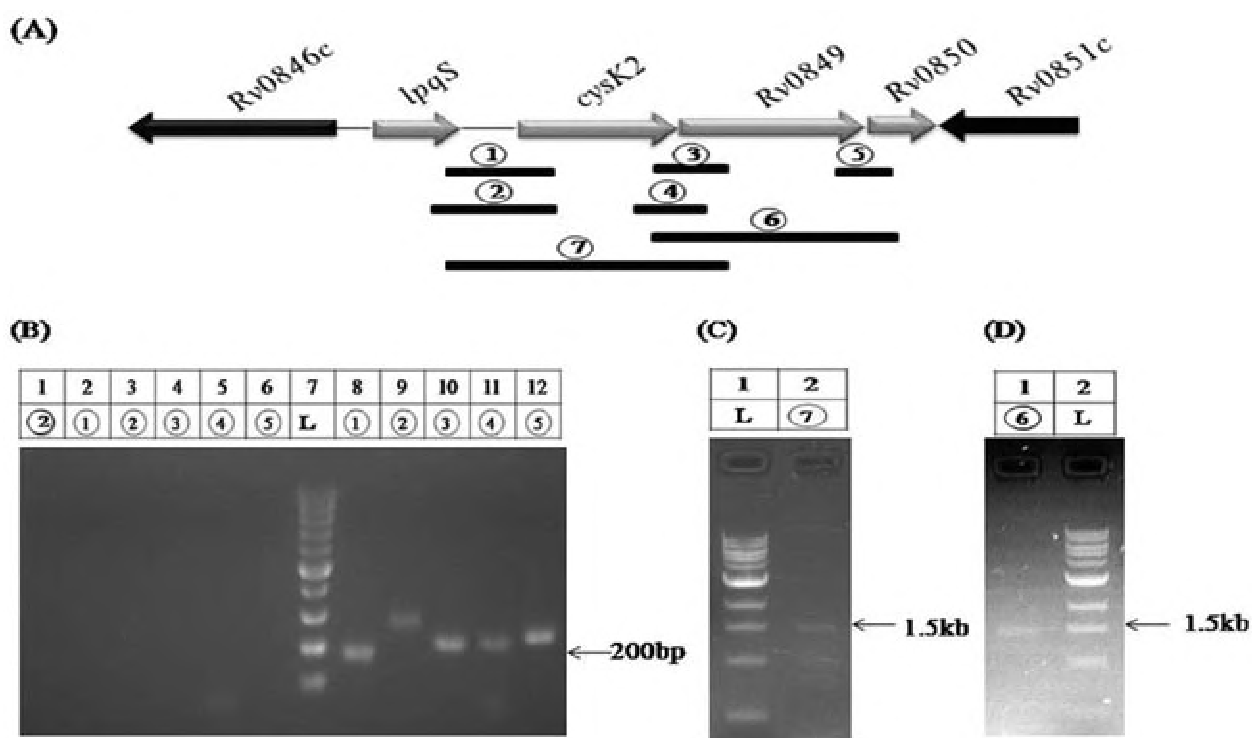

Fig. 2.19: RT-PCR to establish cotranscription of *lpqS*, *cysK2*, Rv0849 and Rv0850.

Total RNA from copper induced *M. tuberculosis* culture was analysed by RT-PCR. Suitable primers were used to amplify the intergenic regions between the gene cluster *lpqS*-Rv0850 using cDNA as template.

(A) Blue arrows represent the genes of the *lpqS* operon. Black bars represents the PCR amplified intergenic regions of the *lpqS* operon using cDNA synthesized from *M. tuberculosis* total RNA. Black bars are numbered on top to denote the intergenic regions amplified by RT-PCR. Primer pairs used to amplify the intergenic regions are listed in table 3.

(B) RT-PCR analysis of the intergenic regions of the *lpqS*-Rv0850 operon. Top row represents the lane numbers and numbers in the bottom row represent the corresponding intergenic region. Lane 1: PCR amplification of intergenic region 2 using RNA as template. Lane 2 - 6: RT -ve controls (Reverse transcriptase enzyme omitted during RT Reactions) for intergenic regions 1-5; Lane 7 (L): 100bp ladder Lane 8-12: Transcripts for intergenic regions numbered 1-5 using cDNA from *M .tuberculosis.*

(C) Lane 1: 1kb ladder; Lane 2: Transcript for the region 7;

(D) Lane 1: Transcript for the region 6; Lane 2: 1kb ladder

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(vii) Global proteomic comparison of *M. tuberculosis* H37Rv and its isogenic *pknE* deletion mutant

Background: TB caused by *M. tuberculosis* survives the hostile immune responses of the host by altering its physiology. These adaptations are governed in *M. tuberculosis* by serine / threonine protein kinases (STPK). STPKs are considered to be drug targets due to their diverse roles in cell division and pathogenesis. Among the eleven STPKs, *pknE* alone was found to inhibit apoptosis. Previously we have shown that PknE plays a role in the adaptation responses encountered within the phagosome. In the current study we have examined the proteomic differences between H37Rv and its isogenic *pknE* deletion mutant. This was executed to identify the role of pknE in regulating the physiology of *M. tuberculosis*.

Aim: (i) to examine the proteomic differences between H37Rv and its isogenic *pknE* deletion mutant during growth in middlebrook 7H9

Methods: The strains *M. tuberculosis* $H_{37}Rv$ (Rv), *M. tuberculosis* $H_{37}Rv\triangle$*pknE* ($\triangle$*pknE*), were grown in middlebrook 7H9 containing albumin dextrose catalase enrichments for growth curve analysis. Late log phase cultures of Rv and $\triangle$*pknE* were adjusted using Mc Farland standards to equal density and whole cell lysates were prepared by sonication. Whole cell lysates were subjected to 2D-gel electrophoresis and the differential protein spots were identified using mass spectrometry (Bruker Daltonik).

Results: Comparison of the proteome profiles of $\triangle$*pknE* (deletion mutant of *pknE*) versus $H_{37}Rv$ (Rv, wild-type) using PDquest™ software showed 945 spots to be differentially expressed. PDquest™ analysis short-listed 75 differentially expressed spots showing three fold cut-offs. Among them, 29 spots were subjected to mass spectroscopic analysis after confirmation with visual examination (Figs. 2.20A and B). These 29 spots after mass spectrometric identification were categorized into seven functional categories based on the information from the database tuberculist (Table 2.8).

In comparison to the proteome profile of Rv, DevR a two- component system involved in various stress responses was unique in $\triangle$*pknE*. The proteins from the functional categories conserved hypotheticals, lipid metabolism and FtsZ, a protein involved in cell division were abundant in the proteome profile of $\triangle$*pknE* in comparison with Rv. Proteins from intermediary metabolism were present in decreased levels. Two proteins pstS1 a phosphate- binding lipoprotein and glyceraldehyde -3- phosphate dehydrogenase, an enzyme involved in glycolysis were absent in the proteome profiles of $\triangle$*pknE* compared with the proteome profile of Rv.

Conclusion: PknE has a role in regulating the metabolism of *M. tuberculosis* that could enable the survival in hostile environments.

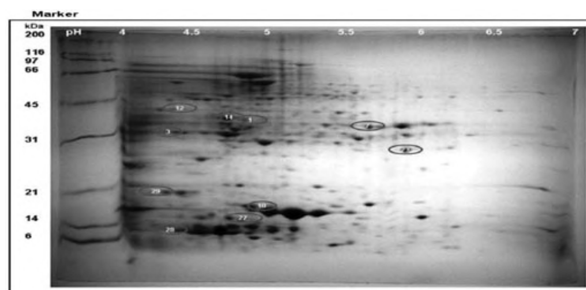

Fig.2.20A: 2D gel showing proteome profile of Rv

Whole cell extracts prepared from cultures grown in Middlebrook 7H9 were subjected to 2D gel electrophoresis and coomassie brilliant blue R250 stained. The red circles depict spots that were analyzed by mass spectrometry. Approximate pH values are labeled above the gel. This is a representative picture from a quadriplicate gel.

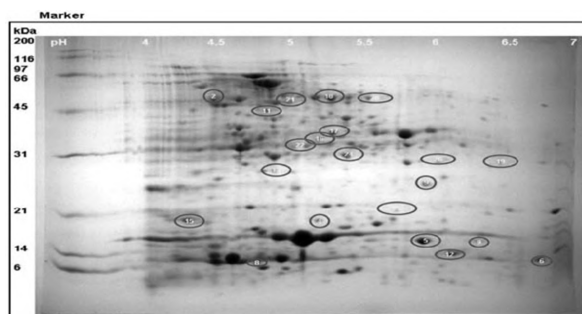

Fig. 2.20B: 2D gel showing proteome profile of △*pknE*

Whole cell extracts prepared from cultures grown in Middlebrook 7H9 were subjected to 2D gel electrophoresis and coomassie brilliant blue R250 stained. The red circles depict spots that were analyzed by mass spectrometry. Approximate pH values are labeled above the gel. This is a representative picture from a quadriplicate gel.

Table 2.8: Details of protein spots from the 2D gels of Rv and *pknE* during growth in middlebrook 7H9 subjected to mass spectrometry

Spot No.	Gene name	Accession No	Mascot Score	Sequence coverage (%)	Theoretical pI/kDa	Expression
Group 1 : Cell wall and cell processes						
1	Periplasmic phosphate binding lipoprotein pstS1	Rv0934	52	14	5.14/38	Absent in ”pknE
2	Cell division protein FtsZ	Rv2150c	62	27	4.55/38	High in ”pknE
Group 2 : Conserved hypotheticals						
3	27 kDa antigen Cfp30B	Rv0577	54	22	4.41/27	Absent in ”pknE
4	Universal stress protein	Rv3788	112	50	5.12/17	High in ”pknE
5	Iron regulated conserved hypothetical protein TB15.3	Rv1636	113	66	5.51/15	High in ”pknE
6	Hypothetical protein	Rv2302	55	37	6.02/8.5	Absent in Rv
7	Conserved hypothetical protein TB31.7	Rv2623	130	48	5.46/31	High in Rv
8	Hypoxic response protein 1	Rv2626c	104	61	4.96/15	Absent in Rv
Group 3: Information pathways						
9	Elongation factor P	Rv2534c	68	24	5.54/20	High in ”pknE
10	Transcription elongation factor greA	Rv1080c	107	61	4.9/17	Absent in ”pknE
Group 4: Intermediary metabolism						
11	Probable NAD(P) transhydrogenase (subunit alpha) PNTAA	Rv0155	123	36	4.9/37	Absent in Rv
12	Glyceraldehyde-3-phosphate dehydrogenase	Rv1436	71	17	5.19/36	Absent in ”pknE
13	dTDP-4-dehydro-rhamnose 3,5-epimerase	Rv3465	58	32	4.93/22	High in Rv
14	Malate dehydrogenase	Rv1240	69	36	4.65/34	High in Rv
15	Probable Tryptophan synthase alpha chain	Rv1613	80	40	4.94/27	High in Rv
16	Phosphoribosylaminoimidazole-succino -carboxamide synthase	Rv0780	133	45	5.1/33	High in ”pknE
17	Probable 3-Hydroxyacyl-thioester dehydratase	Rv3389c	82	24	5.17/30	High in Rv
Group 5 : Lipid metabolism						
18.	3-oxoacyl-[acyl-carrier- protein] synthase 1	Rv2245	59	21	5.11/43	High in ”pknE
19	Probable enoyl-CoA hydratase echA6	Rv0905	106	39	5.97/26	Absent in Rv
20	3-oxoacyl-[acyl-carrier-protein] synthase 2	Rv2246	95	24	5.27/44	Absent in Rv
21	Probable acetyl-CoA acetyltransferase	Rv1323	105	40	4.91/40	High in ”pknE
22	Probable enoyl-CoA hydratase	Rv0222	66	26	5.06/27	High in Rv
23	Probable enoyl-CoA hydratase echA3	Rv0632c	68	27	5.52/24	High in Rv
Group 6: Regulatory proteins						
24	Iron-dependent repressor ideR	Rv2711	95	39	5.2/25	High in ”pknE
25	Probable transcription factor	Rv3583c	66	33	5.49/17	High in ”pknE
26	Transcriptional regulatory protein devR (dosR)	Rv3133c	76	34	5.62/23	Absent in Rv
Group 7 : Virulence, detoxification and adaptation						
27	Heat shock protein HSPX	Rv2031c	53	36	5.0/16	High in Rv
28	10 kDa chaperonin	Rv3418c	125	82	4.62/10	Absent in ”pknE
29	Probable thiol peroxidase	Rv1932	82	52	4.36/17	Absent in ”pknE

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(viii) Identifying the substrates for signal recognition particle pathway proteins

Background: The bacterial signal recognition partricle (SRP) system plays an important role in the biosynthetic pathway of many inner membrane proteins. In bacteria, most of the proteins are exported to cytoplasmic membrane via two pathways, general secretory pathway or SRP pathway. The general secretory pathway is post translational targeting machinery used by a variety of exported proteins, whereas the SRP functions co-translationally to target subsets of proteins whose final destination is the cytoplasmic membrane. *M. tuberculosis* SRP pathway consists of two proteins FfH, FtsY and an RNA subunit 4.5s RNA. The present study focuses on identification of the substrates for SRP proteins, FfH and FtsY of *M. tuberculosis*.

Aim: (I) to identify the protein interacting partners for SRP such as FfH and FtsY of *M. tuberculosis*

Results: Protein expression: *FfH* of *M. tuberculosis* was previously cloned in pBAD vector and transformed in *E. coli* BL21 cells. The construct was revived and over-expressed in LB broth with 1µl/ml Carbenicillin (50mg/ml) and 20% of L-Arabinose (1µl/ml) was added as an inducer.

FtsY of *M. tuberculosis* was previously cloned in pRSETb vector and transformed in *E. coli* BL21 cells. The construct was revived and over-expressed in LB broth with 1µl/ml Carbenicillin (50mg/ml) and 100mm IPTG (1µl/ml) was added as an inducer.

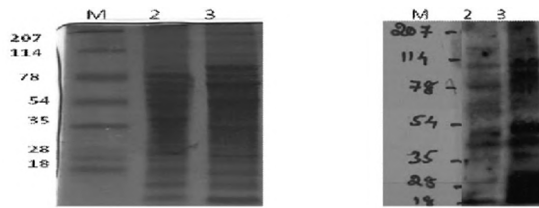
Protein Purification: The over-expressed FfH and FtsY were purified using nickel affinity chromatography and His-tag recombinant proteins were eluted using 200mM Imidazole. The resolution was obtained in SDS-PAGE and confirmed with western blotting. FfH protein was pufiried at 54kDa and FtsY protein was purified at 80kDa and correlates with its molecular weight.

Protein-protein interaction: To investigate the protein-protein interaction, the *M. tuberculosis* H37Rv was grown using 7H9 medium at 37°C. The cells were sonicated to extract the cytoplasmic and crude membrane proteins. These cytoplasmic and crude membrane proteins were used as prey proteins and the recombinant FfH and FtsY were used as bait proteins. Far western blotting (FWB) was derived from the standard western blot method to detect proteinprotein interactions *in vitro*. In FWB, the prey proteins are firstly separated by SDS-PAGE and transferred to PVDF membrane. The proteins in the membrane are then denatured and renatured. The membrane is then blocked and probed with purified bait protein(s). Finally, the membrane is probed with Anti-His antibody and detected using chemiluminescense.

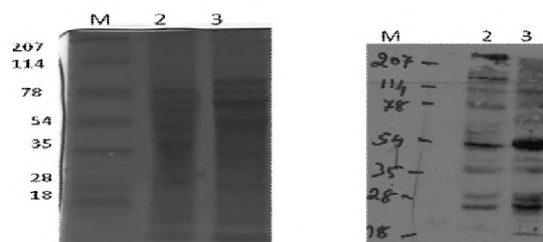
MALDI-TOF MS analysis: Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) peptide mass fingerprint spectrometry analysis was performed to identify the interacting proteins. The interacting proteins were trypsin digested and spotted onto a sample plate for MALDI-TOF MS analysis. The proteins were identified through a search of the NCBI non-redundant database using Mascot (Matrix Science) software.

FfH & FtsY Protein interactions: In order to identify the FfH and FtsY interacting proteins using FWB, the prey proteins were separated using SDS-PAGE and Tricine-PAGE (for identifying low and high molecular weight proteins). FfH and FtsY interacting proteins were then identified by incubating with recombinant His tagged FfH and FtsY respectively. Results from FWB shows that FfH is interacting with 7 different proteins and FtsY is interacting with 6 different proteins of *M. tuberculosis.* The interacting proteins are subjected to MALDI-TOF MS analysis for protein identification.

FfH interacting proteins are identified as PkS, pyruate carboxylase PcA, fatty acid AMP-synthase FAD-26, 30s ribosomal protein S2 RpsB, superoxide dismutase SodA, heat shock protein HspX and a conserved hypothetical protein Rv2159c (Figs. 2.21A & B). Surprisingly, FtsY interacts with all the FfH interacting proteins except the heat shock protein HspX (Figs. 2.22 A & B).



Figs. 2.21 A & B: Substrates for FfH protein
Lane 2 H37Rv Crude lysate, Lane 3 H37Rv Crude membrane fraction



Figs. 2.22 A & B: Substrates for FtsY protein
Lane 2 H37Rv Crude lysate, Lane 3 H37Rv Crude membrane fraction

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(ix) Whole-Genome sequences of four clinical isolates of *M. tuberculosis* from Tamil Nadu, south India

Background: Prevalence of ancestral strains of *M. tuberculosis* in India has been reported using various genotyping methods. The current genotyping methods have low discriminatory power and the whole-genome sequencing of clinical isolates of *M. tuberculosis* has now become preferred approach for genotyping the clinical isolates.

Aim : (I) to perform whole-genome sequencing of four clinical isolates of *M. tuberculosis* from Tamil Nadu, south India

Method: The paired end sequencing was performed on Illumina HighSeq platform.High quality reads were mapped to the genome of reference strain *M. tuberculosis* H37Rv (NC_000962.2) using CLC bio Genomic Workbench.

Results: The NIRT202 genome was generated by aligning 1.34 million paired reads to the reference genome with mapping coverage between 15-53X. The annotation of the genome revealed 3304 genes/CDS and 156 synonymous and 204 non-synonymous SNPs.

Mapping of 06.07 million paired reads to reference genome resulted in to genomic assembly of NIRT203 strain. The mapping coverage ranged from 15-130X. Annotation of the reference assembled genome revealed 3,795 gene/CDS and presence of 615 synonymous and 829 non-synonymous SNPs (Table 2.9).

Table 2.9: Genomic features of four clinical isolates of *M. tuberculosis* from Tamil Nadu, India

Total reads	1,342,728	6,071,857	3,139,081	1,342,045
Coverages	15-53	15-130	15-741	16-36
Genes / CDS	3,304	3,795	3556	3414
Strcutural variations				
Deletion	10	07	10	12
Insertion	07	1,248	38	09
Complex	05	18	37	08
Synonym.	156	615	330	615
Non-synonm.	204	829	296	829

Mapping of 3.13 million paired reads to reference genome resulted in reference assembly of NIRT204 strain. Mapping coverage ranged between 15-74X. Annotation of the reference assembly revealed 3556 gene/CDS and presence of 330 synonymous and 296 non-synonymous SNPs.

Reference assembly for NIRT206 was generated by mapping 1.34 million paired reads with coverage ranging from 16 to 36X. Annotation of the reference assembly revealed 3414 CDS/genes and presence of 615 synonymous and 829 non-synonymous SNPs.

Conclusion: The whole-genome sequencing of four clinical isolates of *M. tuberculosis*, NIRT202, NIRT203, NIRT204, and NIRT206 from Tamil Nadu state of south India are reported here.

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(x) Bioprospecting of selected lichen and plant species for product development against cancer and TB

Background: Poor compliance of the patient *to* the treatment and the *HIV- TB co-infection* led to the emergence of MDR and XDR-TB making *M. tuberculosis* (Mtb) a significant cause for mortality worldwide. The need of the hour is newer anti-mycobacterial drugs. The aim of study is to test the anti-mycobacterial activity of compounds extracted from Lichen and plant products

Aims: (I) to test the anti mycobacterial activity of compounds extracted from lichens and other plant species by agar dilution method

(ii) to test the antibacterial activity of the compound against the intracellular bacilli using THP1 derived macrophage model of infection

Methods:

Evaluation of antimycobacterial activity

Minimum inhibitory concentration by agar dilution method is used for testing the antimycobacterial activity of these compounds at various dilutions. The agar dilution method uses a standardized inoculum grown on media containing only graded concentrations of the compounds to be tested for antimicrobial activity. Middlebrook 7H10 plates supplemented with the specified concentrations of the test compound (ranging from 0.1mg/ml-0.3mg/ml) were used.7H10 plates were inoculated with standard inoculums of *M. tuberculosis* strains and observed for mycobacterial growth till 8 weeks.The minimal inhibitory concentration (MIC) of the compound has been determined .

Evaluation of Intracellular survival

THP-1 cells cultured in RPMI media was supplemented with 10% Fetal bovine serum. Cells were then pelleted by centrifugation at 2000rpm for 10 minutes, washed and re-suspended in RPMI. The number of viable THP-1 cells per ml of media was then determined using Trypan blue staining before infection. Cells were then seeded in 24 well plates at a concentration of 10^6 cells per well and induced to differentiate into macrophage by incubating the cells in the presence of 50 mM phorbol 12-myristate 13-acetate (PMA) overnight. Non-adherent cells were then washed with RPMI and left for resting for 2-3 days in complete RPMI media containing antibiotics. Cells were then washed twice with RPMI , supplemented with complete RPMI media containing amphotericine alone and infected with $H_{37}Rv$ at an multiplicity of infection (MOI) 1:10. Four hours after infection wells were then washed with Hanke's balanced salt solution to remove the extracellular bacilli and supplemented with fresh media. Mtb infected cells were incubated with three different concentrations of the Furan TE1 compound (0.1mg/ml, 0.2mg/ml and 0.3mg/ml) in triplicates. Infected cells were again supplemented with second dose of the lichen extract on day 4. Cells were then lysed using icecold 1% trypsin in RPMI on day 2, 3, 4, 5, 6 to recover intracellular bacilli and serial dilutions made using RPMI, plated on 7H10 plates supplemented with ADS. Plates were then incubated for 4-6 weeks and the cfu were counted to determine the antibacterial activity of the furan compound.

Results: Five compounds extracted from lichen and plant species were tested for their anti-mycobacterial activity against the laboratory strain, *M. tuberculosis* $H_{37}Rv$ and several other clinical strains by agar dilution method. TE1, a compound extracted and purified from lichen exhibited anti-mycobacterial activity against $H_{37}Rv$ and MIC was determined to be 0.15 mg/ml. The anti-mycobacterial activity of the compound was then tested using clinical strains of *M.tb* $H_{37}Rv$ isolated from TB patients. Six clinical strains including three clinical strains exhibiting resistance to RPM and INH (MDR) were used. The compound TE1 exhibited growth inhibition of all the six strains examined and MIC of the compound was determined to be 0.15mg/ml. All experiments were carried out in triplicates (Figs. 2.23 & 2.24).

Compound TE1 was assayed for its toxicity to THP1 cell line by trypan blue staining and the toxicity was minimal. Anti-mycobacterial activity of the compound TE1 against the intracellular bacilli was then studied by macrophage infection. Anti-mycobacterial activity assessed by reduction in CFU of the bacilli in drug treated macrophages compared to that of the untreated macrophages. CFUs counted from Day 2 to Day 6 post infection and treatment exhibited a reduction in CFU of strain tested upto 2 $\log_{10}$CFU at drug concentrations ranging from 0.1mg/ml-0.3mg/ml (Fig. 2.25).

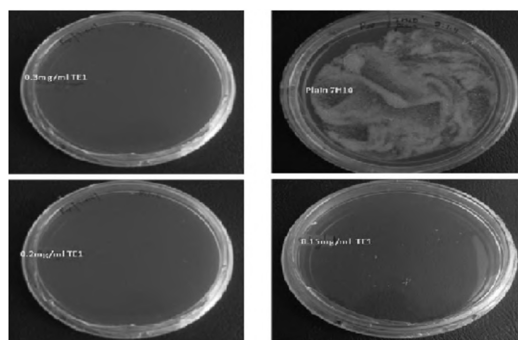

Fig. 2.23: Growth inhibition of $H_{37}Rv$ by the compound TE 1

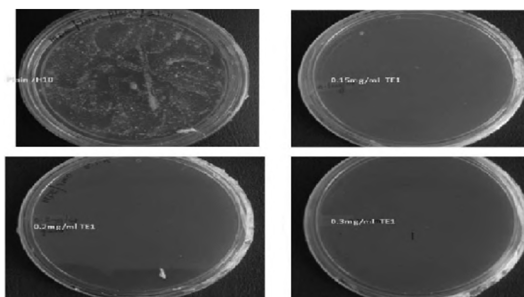

Fig. 2.24: Growth inhibition in MDR strain of *M. tuberculosis* by the compound TE1

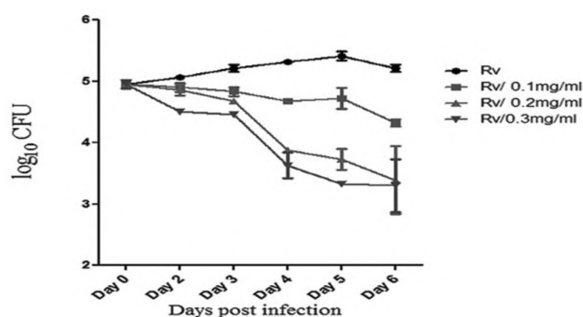

Fig 2.25: Antimycobacterial activity of TE1 on intracellular *M. tuberculosis*

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Clinical strains of *M. tuberculosis* from south India display a wide range of virulence

Background: Virtually all new TB vaccine candidates are tested in animals using the laboratory strains H37Rv or Erdman. However, naturally occurring *M. tuberculosis* infections are caused by strains that are widely different in phenotype and genotype. Very little is known about the characteristics of these clinical isolates in terms of basic biology, virulence and *in vivo* pathogenicity. In this study, we have used a standardized aerosol infection of guinea pigs to compare *in vivo* differences between high and low prevalent clinical strains of *M. tuberculosis* in south India.

Method: Bacterial strains: H37Rv and clinical strains of *M.tuberculosis CS1* CS3 were grown in either Middlebrook 7H9 medium or on Middlebrook 7H10 agar (Difco Laboratories, USA), supplemented with 10% ADC, 0.5% glycerol and 0.05% Tween 80. Cells harvested at mid log phase were washed in PBS, resuspended in PBS containing 15% glycerol and stored frozen at - 80°C till the infection.

Pathogen free 200300 g Dunkin Hartley guinea pigs were housed in stainless steel cages and were provided with ad libitum food and water in a BSLIII facility (National JALMA Institute of Leprosy and Other Mycobacterial Diseases, Agra). All the experimental protocols were reviewed and approved by the animal ethics committee of the institute. Guinea pigs (n = 6) infected with 20 bacilli of virulent *M. tb* H37Rv and clinical strains via the respiratory route in an aerosol chamber (Inhalation Exposure System, Glasscol Inc., IN, USA). 6 weeks after infection animals were euthanized by i.p. injection of Thiopentone sodium (100 mg/kg body weight) (Neon Laboratories Ltd., India). After aseptically dissecting the animals, lung, liver and spleen were examined for gross pathological changes and scored using the Mitchison scoring system. Specific portions of lungs and spleen were then weighed and homogenized separately in 5 ml saline in a Teflon glass homogenizer. Appropriate dilutions of the homogenates were inoculated on to MB7H11 agar plates in duplicates and incubated at 37°C in a CO2 incubator for four weeks. The number of colonies were counted and expressed as log10 CFU/g of tissue.

Results and Discussion:

Based on lesion scores clinical strains could be categorized as follows:

	Lung	Spleen
Naive	No tubercles	No tubercles
H37Rv	Heavy involvement with numerous large tubercles measuring 3–5mm in diameter	Scanty involvement with few large tubercles or numerous small but easily visible tubercles
Clinical strain (CS1)	Minimal involvement with small scanty tubercles	Minimal involvement with one or two large tubercles or moderate number of just visible tubercles
Clinical strain CS2	Moderate involvement with occasional large tubercles (3–5 mm) or more numerous small tubercles (1–2 mm)	Moderate involvement with numerous small tubercles or markedly enlarged spleen plus numerous small tubercles
Clinical strain CS3	Scanty involvement up to four large tubercles or number of small tubercles	Minimal involvement with one or two large tubercles or moderate number of just visible tubercles

Virulence based on *in vitro* growth after aerosol challenge in guinea pigs have shown that all the clinical strains from south India are less virulent compared to H37Rv standard. The most prevalent strain in south India, EAI shows a variable virulence in guinea pigs. The strain CS1 which is EAI3 show a lower virulence compared to other strains infected. Where as CS2 which is EAI5 shows higher virulence among the strains tested. This shows a variable virulence even in a single genogroup.

The most transmissible and virulent genotype Beijing is less prevalent in south India and they are less virulent compared to H37Rv and CS2 clinical strain. Lesion score for CS2 strain is more for spleen compared to H37Rv but the CFU is less than H37Rv this might be due to the fact that the infection is getting cornered due to granuloma formation (Figs. 2.26-2.29).

Conclusion: The clinical isolates from south India produced an unexpectedly wide range in severity of pulmonary and extrapulmonary lesions compared to laboratory H37Rv strain.

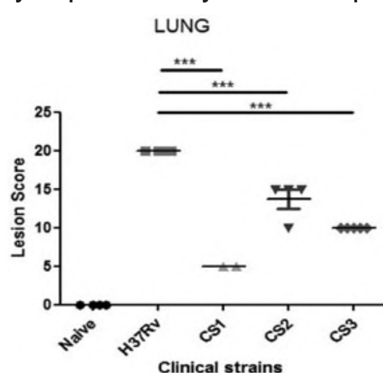

Fig. 2.26: Lesion score in lungs

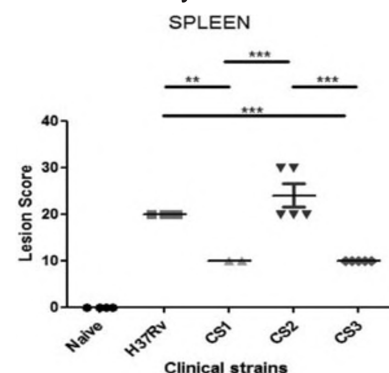

Fig. 2.27: Lesion score in spleen

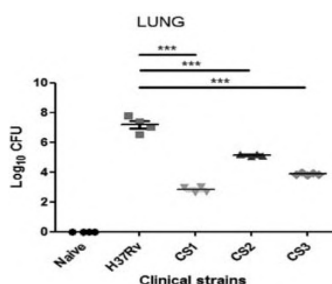

Fig. 2.28 : Log $_{10}$ CFUs of the *M. tuberculosis* strains In lung

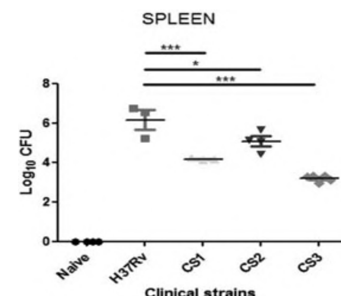

Fig. 2.29: Log $_{10}$ Colony forming units of *M. tuberculosis* strains in spleen

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(x) Role of Chemokine, DC-SIGN and TLR gene variants on immunity to TB

Background: Invasion of the host by microbial pathogens causes activation of the innate immune response (first line defense) and triggers the secretion of various chemokines and cytokines and initiation of adaptive immunity. Chemokine, DC-SIGN and TLR gene polymorphisms have been studied and shown to be associated with susceptibility or resistance to various infectious diseases.

Aims: (i) to find out whether Chemokine, DC-SIGN and TLR gene polymorphisms are associated with susceptibility or resistance to TB

(ii) to understand the role played by these gene variants on the innate and adaptive immunity to TB

Under this main study, the following studies are being carried out:

I. Regulatory role of chemokine gene polymorphisms on chemokine expression in PTB

Background: Polymorphisms in the chemokine genes have been shown to regulate the production of chemokines. In the present study, the regulatory role of various chemokine gene polymorphic variants on chemokine expression was studied in newly recruited PTB and healthy volunteers.

Aim: (i) to understand the regulatory role of various chemokine gene polymorphisms on chemokine expression

Methods: The study was carried out with newly recruited PTB patients (n=60) and healthy volunteers (n=60). The chemokine levels of MCP-1, MIP-1a , MIP-1ß , RANTES, IP-10 and SDF-1 were estimated in the 72 hrs culture supernatants of peripheral blood mononuclear cells stimulated with *M.tuberculosis* antigen and the cells were used for determination of intracellular chemokine positive cells. Chemokine gene polymorphisms were determined using the DNA extracted from the white cells of the patients and healthy controls. Chemokine gene polymorphisms will be correlated with the level of chemokines as well as intracellular chemokine positive cells.

Results: Results are being analysed.

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II. Effect of vitamin D_3 on CD14, CD206, CD209, Beclin and ATG-5 expression in macrophages infected with live *M. tuberculosis*

Background: Immune responses after mycobacterial infection are initiated by recognition of mycobacterial components through various host receptors. Phagocytosis and subsequent induction of autophagy plays key roles to eliminate the intracellular pathogen. Vitamin D_3 is known to have various immunomodulatory roles on innate immunity; it enhances macrophage phagocytosis by up-regulating the expression of pattern recognition receptors and induces the expression of antimicrobial peptides.

Aim: (i) to understand the effect of vitamin D_3 on the expression of CD14, CD206, CD209,Beclin and ATG-5 in monocyte/ macrophages in PTB

Methods: The study was carried out in 20 PTB patients and 20 HC subject. Peripheral blood mononuclear cells were cultured for 72hrs with live *M. tuberculosis* and its CFA in the presence and absence of vitamin D_3. The non-adherent cells were aspirated gently and the adherent 72hrs old monocytes/ macrophages were used for RNA extraction and cDNA

synthesis. The relative quantification of target genes such as CD14, CD206, Beclin, ATG-5 and the housekeeping gene, GAPDH was done using real-time PCR with TaqMan assay primers and probes.

Results: The results are being analysed.

(Contact person: Dr. P. Selvaraj, email: selvarajp@nirt.res.in)

III. Regulatory role of vitamin D receptor gene variants on vitamin D_3 modulated intracellular chemokines in PTB

Background: Our earlier studies revealed the regulatory role of VDR gene variants on various immune functions in PTB. We have reported that vitamin D_3 significantly alter the various extracellular chemokine levels in the cell culture supernatants of mononuclear cells stimulated with *M. tuberculosis* antigens. In the present study, the regulatory role of VDR gene variants on vitamin D_3 modulated intracellular chemokine expression is studied in PTB.

Aim: (i) to find out the regulatory role of VDR gene promoter region polymorphism (Cdx-2) and 3' untranslated region polymorphisms (TaqI and BsmI) on vitamin D_3 modulated intracellular chemokine expression in PTB

Methods: The VDR polymorphisms were determined in 50 HCs and 50 PTB patients. Cdx-2 polymorphism was studied by PCR with allele-specific primers. TaqI and BsmI polymorphisms were studied by PCR based RFLP method. Whole blood cells were cultured for 72hrs with live *M. tuberculosis* and its antigen with or without vitamin D_3 at a concentration1X10^{-7}M. After 72hrs, the cells were processed for immunostaining using specific monoclonal antibodies against CD3, CD4 and CD8 surface markers and intracellular chemokines which include MCP-1, MIP-1á, MIP-1â, RANTES and IP-10 and analysed in flow cytometry.

Results: The results are being analysed.

(Contact person: Dr. P. Selvaraj, email: selvarajp@nirt.res.in)

(xi) Neutrophil mediated innate immune responses in TB: *In vitro* studies to understand the interaction of mycobacterial vaccine strains with neutrophils.

Background: There is an increasing support to the hypothesis that neutrophils are the primary cells involved in early inflammatory host response during mycobacterial infections. In our previous study, we have shown modulation of immune responses in normal and TB neutrophils by clinical strains of MTB (S7 and S10). However, very little is known about the potential of various vaccine strains to stimulate neutrophils. As neutrophils are the first cells to get exposed to any antigen and generate early immune response, their interaction with vaccine strains will help us to understand the exact nature of protective immune response.

Aim: (i) to compare the differential capacity of vaccine, *M. bovis* Bacillus Calmette Guerin *(*BCG) and *M. indicus pranii* (Mw) and laboratory strain H37Rv to activate and enhance neutrophil functions. The expression of phenotypic markers like Fc ? receptor, TLR, chemokine receptor and pro-inflammatory cytokines were studied in infected neutrophils

Methods: Purified neutrophils were infected with mycobacterial vaccine strains at MOI of 3 and the initial activation was studied at early time point of 4 hrs. Uninfected neutrophils served as negative control and PMA stimulated cells were used as positive control. Cell phenotyping was done by flowcytometry. The inflammatory cytokines like TNF- and IFN- were measured in infected neutrophil culture supernatants (Nu sups) using commercial ELISA kits.

To understand the paracrine role of neutrophils, the Nu sups were used to stimulate monocytes and T helper cells.

Results: Increased expression of CD32, CD64, TLR4 and CXCR3 was observed in H37Rv infected neutrophils (Fig. 2.30). Among the vaccine strains, BCG increased the expression of only CD32 on neutrophils while Mw was comparatively ineffective. An increased TNF- was observed only in H37Rv infected neutrophils while vaccine strains didn't have profound effect on the release of TNF- (Fig. 2.31). None of the strains was able to modulate the secretion of the major pro-inflammatory cytokine IFN- by neutrophils. The secretory molecules from all infected neutrophils (Nu sups) increased the expression of CCR5 on monocytes whereas only H37Rv infected supernatant increased the expression of CCR7 on monocytes and CD69 on T-cells (Fig. 2.32).

Conclusion: Thus, H37Rv was more effective in activating neutrophils and in turn stimulating monocytes and T-cells. In comparison, vaccine strains were less effective in modulating neutrophil functions.

(Contact person: Dr.D. Sulochana, email: dsulochana@nirt.res.in)

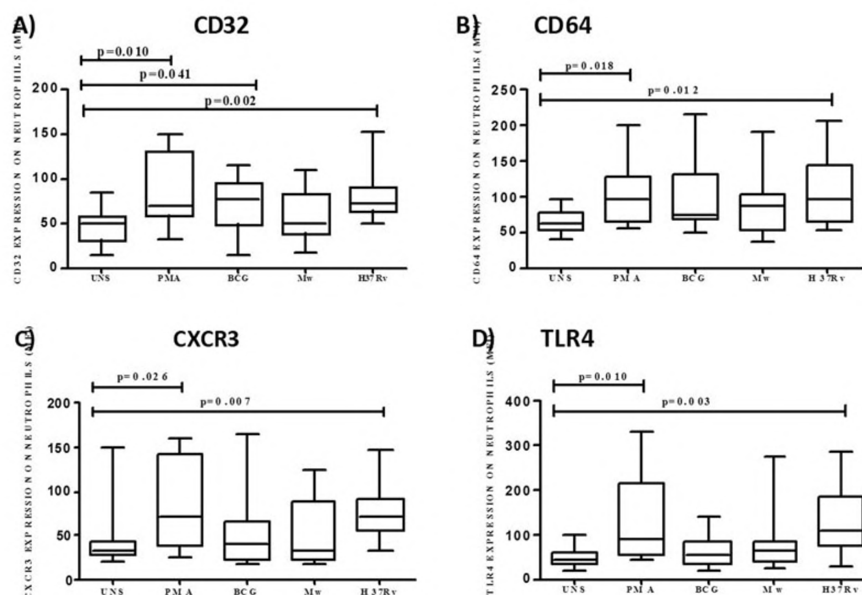

Fig. 2.30: Expression of cell surface signaling receptors

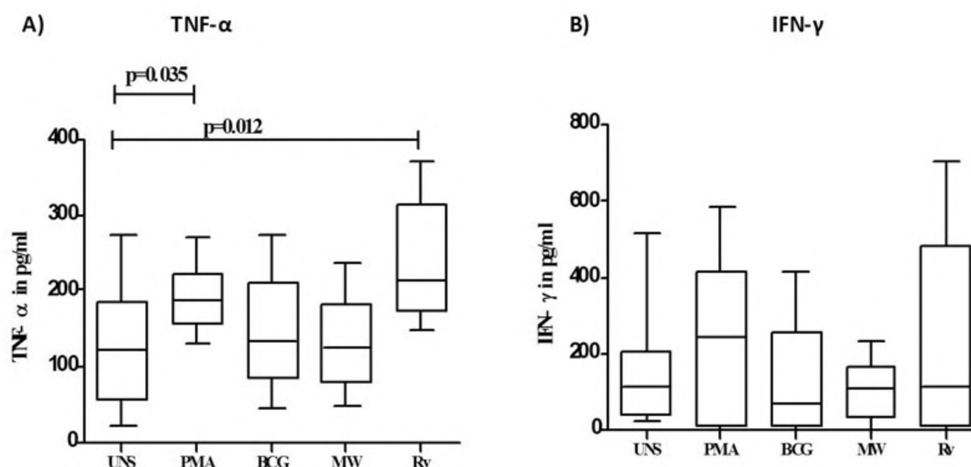

Fig. 2.31: Secretion of pro-inflammatory cytokines

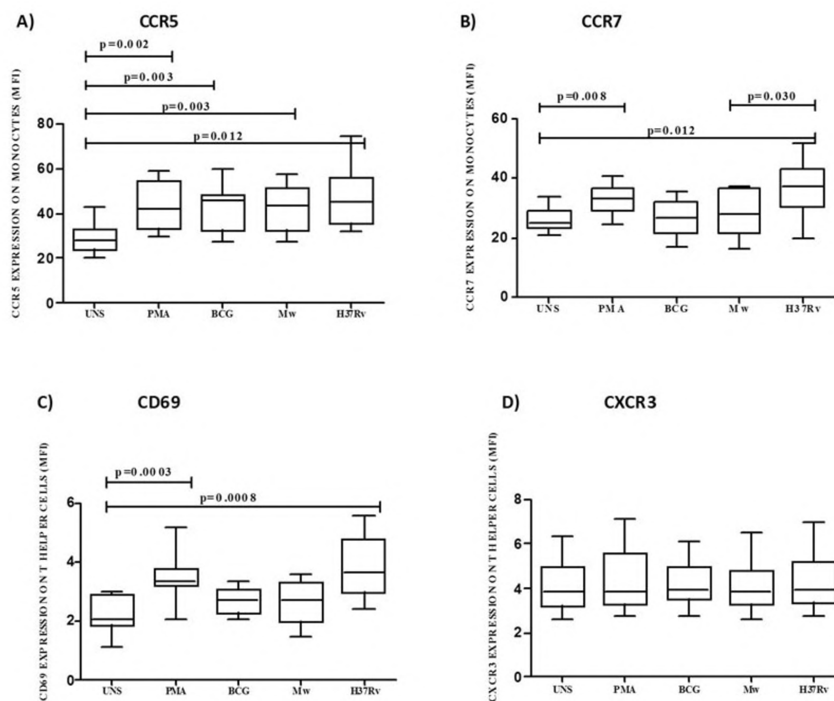

Fig. 2.32: Effect of neutrophil secretory products on monocytes & CD4+ Th cells

(xii) *In vitro* modulation of signaling receptors on neutrophils infected with *M. tuberculosis* strains

Background: Neutrophils are the primary cells that contribute to initial defense against mycobacteria and directly influence macrophages and T-lymphocytes. Thus the immune response created by neutrophils is of primary importance in initiating downstream immune signaling to overcome the mycobacterial infection.

Aim: (i) to understand the differential capacity of *M. tuberculosis* strains to activate and modulate cell surface mediated neutrophil signaling and functions

Methods: Dextran purified neutrophils from 20 healthy volunteers were stimulated with PMA and infected with clinical strains (S7 & S10) and laboratory strain H37Rv and cultured for 4 and 20 hrs. The cultured cells were analyzed for activation markers (CD11B and CD66B) and signaling markers like TLRs (TLR-1,-2,-4), chemokine receptors (CCR-1, CXCR-1,-2) and FC receptors (CD16, CD32, CD64) by flow cytometry. The results are shown as mean of percentage positive cells expressing each marker.

Results: Neutrophils infected with H37Rv showed significant increase ($p<0.05$ to 0.0001) in expression of all activation and signaling markers (except CD16) at both the time points (Figs. 2.33-2.35). At early time point, S10 infected neutrophils showed significant increase ($p<0.05$ to 0.001) in expression of most of these markers except CD16, CXCR1, TLR1 and -4, while S7 showed significant increase only in CD32 ($p=0.01$), CD64 ($p=0.0009$) and CD66B ($p=0.01$). At late time point, clinical strains could not alter the expression levels of these markers on neutrophils.

Conclusion: The increased expression of various signaling receptors along with the activation markers on H37Rv infected neutrophils indicated its strong capacity to activate and initialize the immune response. Compared to H37Rv, clinical strains modulated neutrophil functions marginally.

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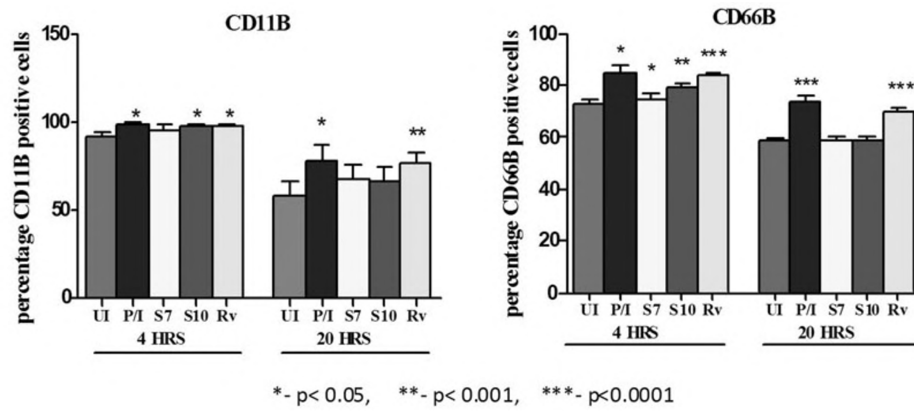


*- p< 0.05, **- p< 0.001, ***- p<0.0001

Fig. 2.33: Expression of activation markers

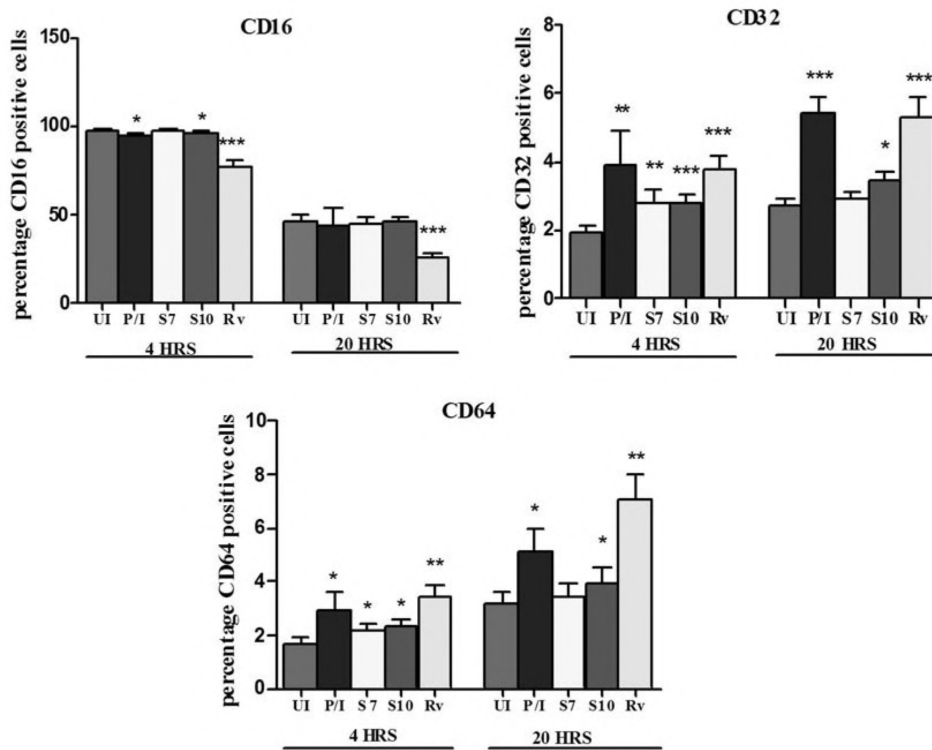
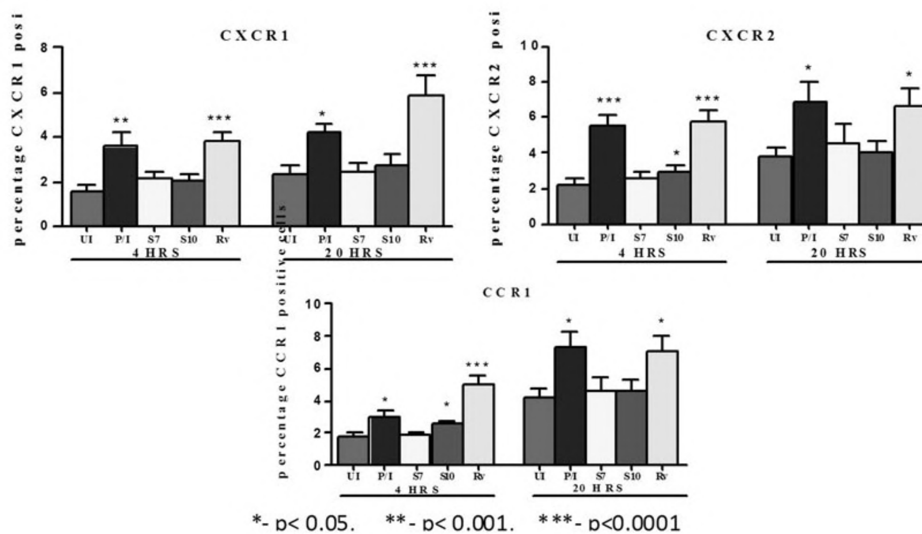


Fig. 2.34: Expression of Fc ? receptors



*- p< 0.05, **- p< 0.001, ***- p<0.0001

Fig. 2.35: Expression of chemokine receptors

HIV-TB Laboratory

Overview: HIV/AIDS laboratory in the Department of Clinical Research has been in the forefront providing high quality diagnostic and research support for various HIV and HIV/TB clinical trials and research projects conducted by the NIRT. The workload of the department is managed by a professional team of 2 scientists, 5 technical officers, 5 technical assistants, 2 technicians and 2 support staff who have permanent tenure and 1 technical officer,1 technical assistant, and a senior research fellow who work for the institute in fixed tenure. At present, three research scholars are pursuing research for a Ph.D. degree in the HIV/AIDS Laboratory.

The laboratory has various dedicated sections like the Serology lab, Flow cytometry facility, Viral load lab, Cellular Immunology lab, Molecular Biology Lab, HIV Drug Resistance testing lab and Virology lab, that function to carry out routine and research activities pertaining to HIV/AIDS. The Laboratory has been accredited by International Agencies like the NIH and WHO, and participates in several External Quality Assurance programs such as NIH-VQA and CDC-GAP for HIV-1 DNA PCR, NIH-VQA and RCPA-QAP for HIV-1 viral load assay, NIH-VQA and WHO for HIV-1 drug resistance genotyping, QASI (NARI) for CD4 and HIV Serology EQAS with NARI for diagnostic ELISA. The lab has been functioning as the Regional Reference Laboratory and centralized testing facility for the National AIDS Control Organization for two National Programs viz. The early infant diagnosis program and the second line ART program. The HIV/AIDS laboratory is also a National Reference Laboratory for WHO for HIV-1 Drug Resistance Genotyping.

The focus of research in the laboratory include immunopathogenesis of HIV/TB co-infection, molecular characterization of subtype C strains circulating in the local population and identification of novel compounds with anti-HIV activity. The laboratory has also been imparting project training and internships to selected eligible undergraduate and postgraduate students from different universities.

Studies in progress

(i) Molecular characterization of the envelope gene of HIV-1 clinical isolates

The HIV *env* gene is around 2.5 kb long and codes for around 850 amino acids. The primary *env* product is the protein gp160, which gets cleaved to gp120 and gp41 in the endoplasmatic reticulum by the cellular protease furin. Gp120 is a 120 kilodalton glycoprotein exposed on the surface of the HIV envelope and is essential for virus entry into cells as it plays a vital role in attachment to specific cell surface receptors. Hence gp120 was one of the first and most important targets of HIV vaccine research. However, HIV-1 envelope diversity remains a significant challenge for the development of an efficacious vaccine. The diversity of *env* has been shown to increase by 1-2% per year in HIV-1 group M and the variable units are notable for rapid changes in amino acid sequence length. While it has been reported that an increase in gp120 variability results in significantly elevated levels of viral replication, indicating an increase in viral fitness, the evolutionary forces that shape the diversity of envelope are incompletely understood.

HIV-1 subtype C envelope in particular shows significant differences and unique characteristics. The present study has been undertaken to understand the characteristics of the envelope gene of HIV-1 subtype C strains involved in the epidemic in the Indian subcontinent. Full-length *env* genes of HIV were amplified from co-cultured PBMC obtained from HIV-1-infected subjects in various stages of HIV disease using nested PCR

and sequenced completely. The sequences were subjected to phylogenetic analysis, N-linked glycosylation analysis, co-receptor usage/tropism analysis and presence of resistance mutations to entry Inhibitors.

Phylogenetic analysis revealed that all sequences generated in this study clustered on a distinct branch within the subtype C, along with other Indian subtype C sequences, indicating that the envelope sequences analyzed in this study exhibited common amino acid signatures and low genetic distances, thus showing a level of genetic relatedness. Co-receptor usage/tropism of the clinical isolates was predicted using the online tool Geno2Pheno designed to predict co-receptor usage from the V3 loop sequence. All isolates showed characteristics of exclusive use of CCR5 as the co-receptor.

N-linked glycosylation analysis was performed using the *N-Glycosite* online tool. The positions of the sites were referenced using the HXB2 prototype sequence. The NLG frequencies were found to vary greatly in number and position in the different envelopes. Frequency of glycosylation was higher in the V1, V2 and V3 loops, and very low in the V4 and V5 loops. No known resistance mutations were identified in any of the sequences, indicating the absence of primary resistance in the population tested, this probably could represent a similar scenario in the Indian population which is naive to this class of drugs.

In brief, the present study has thrown light on the uniqueness of the envelope gene of HIV isolates circulating in the Indian population, the pattern of exclusive CCR5 co-receptor usage, lower frequency of glycosylation, and the absence of primary resistance to the class of antiretroviral drugs classified as entry inhibitors. In depth analysis of the characteristics of a larger number envelope genes of the clinical isolates from infected individuals in this population will provide useful insights about the nature of the disease characterizing the subtype of virus prevalent in the local population.

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(ii) Characterization of anti-HIV activity of transitmycin

A novel brominated compound isolated from an actinomycete obtained from the coral reef ecosystem in Rameshwaram showing anti-mycobacterial activity was also evaluated for its anti-HIV activity. Briefly, healthy donor PBMC activated through PHA stimulation for 72 hours were incubated with 100$TCID_{50}$ of the a laboratory adapted strain of HIV (HIV-1 IIIB) in the presence of absence of varying concentrations of the test (0.001µg/ml, 0.01µg/ml, 0.1µg/ml, 1.0µg/ml, 5.0µg/ml and 10.0µg/ml). Control cultures were set up without compound. Cultures were maintained for 7 days and HIV-1 p24 antigen levels were measured in culture supernatants. Anti-HIV activity was identified by a reduction in p24 levels in cultures containing the compound as compared to control cultures. >90% inhibition was observed at a concentration of 0.1µg/ml.

Activity of transitmycin on different HIV-1 subtypes was also examined. Activated donor PBMC were infected with 100$TCID_{50}$ of primary clinical isolates representing different HIV-1 clades (clades A, B, C, D, E, A/E), as well as NVP resistant and AZT resistant strains, in the presence of test compound (the viruses were obtained from the NIH AIDS Reagent and Reference Program). >90% reduction in p24 antigen level was observed with all primary isolates when the compound was added at a concentration of 1.0µg/ml.

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(iii) Genetic characterization of HIV-type 1 subtype C strains circulating in south India

The HIV-1 subtypes display genetic variations that can potentially alter the functions of several proteins and thereby influence HIV-1 mediated pathogenesis. A study was undertaken with the objective to clone and characterize the full genome of HIV-1 isolates circulating in the south Indian population, as this understanding would further our knowledge on the genomic heterogeneity and adaptive evolution of the circulating viral strains in the country. Ten HIV-1 seropositive individuals naive to antiretroviral treatment attending the HIV clinic at the NIRT, Chennai, were recruited for the study.

Patient PBMC were co-cultured with an equal number of PHA-stimulated PBMC obtained from HIV negative healthy donors depleted of CD8 cells, for 2 weeks. Genomic DNA was extracted from cultured cells and full-length provirus was amplified by nested PCR as two fragments. The fragments were cloned into a commercial plasmid vector and recombinant clones containing full-length inserts were identified by restriction analysis. Full-length HIV genomes of 6 such clones were sequenced. Sequences of the viral clones were aligned with full-length reference sequences of several Group M viruses obtained from the Los Alamos sequence database using CLUSTAL X.

Sequences of the six clone sequences were analyzed for variability in each of the individual viral genes and proteins. The nucleotide sequences were conceptually translated into the respective protein sequences and analyzed for sequence, presence of resistance mutations for known drugs, variability, etc. Preliminary analysis revealed the following features. The LTR sequence of five of the six clones showed the presence of three NF-κB sites, while one had only two NF-κB sites with a deletion of the III-Nf-κB site. All Tat sequences had the CS motif instead of the CC motif which has been previously demonstrated to render the protein defective in its chemokine property. Rev genes of 4 clones code for a 107 amino acid protein while that of the other 2 clones code for a 100 amino acid protein. One of the clones had mutations conferring resistance to nevirapine in the RT gene, in spite of a recorded history of no previous exposure to antiretroviral drugs. Except for one clone which revealed characteristics of CXCR4 usage/X4 tropism, all other clones had sequence motifs that predict CCR5 usage/R5 tropism.

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Services offered to National Programs

(iv) Molecular diagnosis of HIV infection in infants for the early infant diagnosis Program of NACO

For the National early infant diagnosis Program, 1925 blood samples from infants (aged 6 weeks to 18 months) born to HIV-infected women, were received in the form of dried blood spots from the states of Tamil Nadu, Kerala and Pondicherry during the period April 2012 to March 2013. Of the 1925 samples received for testing, almost 50% were collected from children <2 months of age. The samples were tested in the HIV/AIDS Laboratory by PCR using the Roche Amplicor v1.5 assay. The percentage positivity was ~3%.

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(v) Plasma viral load testing for the Second Line ART Program of NACO

Plasma viral load was tested for 931 HIV-1-infected individuals enrolled for first-line anti-retroviral treatment under the National AIDS Control Program, experiencing clinical symptoms of treatment failure, in order to confirm virological failure and subsequent eligibility for second-line anti-retroviral therapy. Measurement of viral load was performed using the COBAS Amplicor HIV-1 Monitor kit v1.5 (Roche Diagnostics).

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3

SOCIOLOGICAL STUDIES

Department of Social & Behavioral Research

Overview: The department was started in August 2012 and has been conducting intervention studies with vulnerable and hard to reach populations which include men having sex with men (MSM), mothers living with HIV (MLH), migrants (brick kiln workers) and TB patients with alcohol use disorders (AUD). The major achievements are that we have been able to disseminate research findings to stakeholders and policy makers. Our Community Advisory Board (CAB) and our training programs have helped in ongoing dissemination of our research activities. We have also been successful in our training programmes to various target groups like PG students of social work, medical sociology, and NSS programme officers and students from various colleges in Tamil Nadu. We have so far trained 850 NSS programme officers and PG teachers. We have also been involved in community awareness programmes on TB through puppet show and folk song. We have also prepared IEC materials on TB which include flip charts, posters and pamphlets.

Apart from assessing socio-behavioural aspects and suitability of patients for clinical trials, the department has been involved in rehabilitation programmes for our study participants. This has helped us to go beyond research and work on the felt needs of the community. This has also facilitated networking with NGOs who have partnered with us in our various research activities.

Studies completed:

(i) Health seeking behaviour and awareness of TB among migrants – brick kiln workers A study from Tiruvallur District, Tamil Nadu, India

[Source of funding:USAID through WHO]

Background: Early detection and treatment of TB patients have been key principles of TB control. However this can be a challenge with some hard to reach populations and migrants are one such group. It is necessary to focus on social determinants of TB and the barriers hampering effective TB control among this group.

Aims:
- (i) to understand the knowledge, attitude and perceptions on TB among brick kiln migrant workers
- (ii) to identify the prevalence of chest symptoms and health seeking behaviour among brick kiln workers
- (iii) to find out the perceptions of the health providers in providing treatment and management of TB care with special reference to brick kiln migrant workers

Methods : This was a cross-sectional community based study carried out from August 2011 to June 2012 in Tiruvallur District, southern India which is home to a number of migrant brick kiln workers. A total of 4002 individuals from 65 chambers randomly selected were interviewed to find out the prevalence of chest symptoms and their health seeking behavior patterns. This study was done in phases following a sequential approach with a quantitative phase and qualitative phase.

Results: The total number of chest symptomatic identified among the brick kiln migrants was 377 (9.4%). The prevalence of chest symptoms among male and female was 10.4% and 8.3% ($p<0.05$) and prevalence was found to increase with age $p<$ (0.01). The most significant variables associated with chest symptoms were education, alcohol, smoking and duration of stay in the chamber ($p<0.01$). The most significant factor associated with action taking among the symptomatic was the length of stay in

the chamber (e" 6mths) (OR, 5.5 95% C.I.: 2.3- 13.3).

Conclusion: The findings indicated that social determinants such as age, sex, alcohol, smoking and time spent within the chamber contributed to the development of chest symptoms. Furthermore, care seeking behaviour patterns were also strongly influenced by the duration of stay in one particular place, which is a big challenge to the RNTCP programme considering the mobility of this population.

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(ii) A pilot study to test the feasibility and impact of an intervention for mothers living with HIV/AIDS

Background: This is an outcome of a previously conducted Indo-US study done using a community based approach to design an HIV/AIDS program for MLH. This study had brought out key areas that challenge MLH. This included barriers to accessing care, stigma from health providers, disclosure issues, legal issues, nutrition and coping with the illness. This pilot experimental intervention study was done keeping in mind the felt needs of the MLH and the strategies adopted were based on the suggestions of the MLH. The study findings would help to plan a larger experimental study to assess the impact of the intervention model on the quality of life of MLH.

Aim: The specific objectives of this study are to:

(i) compare the intervention care group and standard care group with respect to physical heath status, mental well being, social and family support, quality of life

Methods: This community-based, prospective, randomized, experimental two-group design randomly assigned 32 MLH to intervention and 32 MLH to a standard program. The intervention program consists of five monthly sessions delivered over five months.

Outline of intervention sessions:

Sharing my experiences - dealing with stigma and disclosure issues, compliance for ART maintaining a balanced family life, nutrition, maintaining a balanced family / social life / parenting, dealing with legal issues / feedback

Results: Eighty one percent of the participants have completed the 3-month assessment and 72% of them have completed the 6-month assessment. Almost 37% of them were below 30 years and 54% among them were working women. It was seen that 63.5% of women had at least one child who is HIV positive. Overall, those who had more than one child who was HIV positive had a higher stigma score and had a lower self-esteem.

They were divided into intervention group and control group (32+32). Heard stigma and enacted stigma showed significant difference between baseline and 3rd month follow-up and the differences were reflected in the total scoring of stigma. A significant difference was also observed between the baseline and 6th month follow-up for depression. No significant association was found between stigma and the demographic variables when adjusted for time.

This pilot study provides the background for a large scale efficacy trial to provide a model replicable intervention for MLH.

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Studies in progress:

(i) HIV prevention through mobile phone technology among male sex workers in India

[Funding source: Indo-US Joint working group on HIV]

Background: This proposal is an outcome from a collaborative intervention done in NIRT with the Harvard medical school to address psychosocial needs of MSM. Our prior formative work revealed that the vast majority of sex workers in India have mobile phones and use these to network with “pimps” (individuals who manage male sex workers (MLSWs) and mediate client interactions), other sex workers, and to schedule sex work clients.

Aims : (i) to fully develop an HIV risk reduction counselling intervention for MLSWs in India, using mobile phone technology.

(ii) to examine the feasibility, acceptability and potential impact of the proposed intervention in a pilot randomized controlled trial

(iii) to assess if potential mediators of intervention differentially change in the intervention group and if these changes are associated with the primary outcome (reduced sexual risk taking).

Methods : The study was initiated in 2011 and will be done in phases.

Phase 1: Intervention Development (completed)

1a. Conducted formative qualitative interviews (N = 40) and focus groups (4 groups with 6-8 participants) to develop the intervention with community and participant inputs. This phase helped to further develop all study procedures, and helped finalize the intervention manual.

1b. This phase included an open-phase pilot with 10 MSM sex workers. This was done to pilot test the study procedures and tests the intervention. Voluntary testing for HIV among open-phase pilot participants at baseline was done. The assessments will be done at 3 months post-intervention and at 6th months.

Phase 2: Intervention – Randomized controlled trial (RCT)

In this phase we will examine, in a pilot RCT, the feasibility, acceptability, and potential impact of the proposed intervention [with 100 MSM sex workers (80 completers: 40 per study site)] on reductions in sexual risk taking behaviour. This phase is to be initiated.

(Contact person: Dr. Beena E Thomas, e.mail: beenathomas@nirt.res.in)

(ii) An experimental study to enhance treatment adherence in TB patients with alcohol use dependence

[Source of funding: Model DOTS project]

Background: This study is an outcome of a previous pilot study conducted during 2009-10 in Chennai Corporation to explore the frequency of alcohol use among TB patients. 29% of the 490 TB patients (all male) consumed alcohol, the prevalence of AUD among them being 52%. The qualitative component of the study highlighted the need of an intervention among TB patients with AUD and a study to test the feasibility and the acceptability for such an intervention. This randomized experimental intervention study has been planned to enhance treatment adherence in TB patients who consume alcohol.

Aims: (i) to enhance treatment adherence of TB patients who consume alcohol by reducing the default ratethrough intervention strategies

(ii) to evaluate the impact of intervention strategies by comparing the treatment adherence of TB patients with disorders related to alcohol use in the experimental area with those TB patients with disorders related to alcohol use in the control area

Methods: This study was carried out in 4 out of 10 zones in Chennai. They were selected based on the prevalence of TB in their respective zones with one high prevalence and low prevalence zone. The study sites included 4 of the 10 corporation zones. All TB patients above 18 years of age diagnosed during the period of were considered for the study. The study is done in phases.

Phase 1: Qualitative phase

This phase included FGDs, in-depth interviews with patients, family members and health providers. The baseline data on total number of patients on different regimens, cure rates, defaulter rates, reasons for default and the number of patients who consume alcohol was obtained from the study sites. Based on the findings, from the situational analysis, an intervention module was designed for the intervention among the TB patients.

Phase 2: Quantitative phase

So far we have screened 1512 TB patients and 569 were recruited. The eligibility criteria included adult e"15 years of age, those who were administered the AUDIT and found to consume alcohol, willing to take part in the study and provide written consent. The participants are assessed at base line and 6th month using a semi structured interview schedule and includes the AUDIT scale to measure alcohol use and the alcohol dependence scale (ADS) to measure alcohol dependence. The interventions for those in the experimental group include individual counseling and cognitive behavior therapy. A group session is also included. Visual aids which explain the adverse effects of alcohol on general well being and with special reference to TB are also utilized during the individual sessions. Patient will be given the intervention at 0, 1, 2 & 4th months.

Outcome measures

The following outcome measures will be compared between the experimental and control zones:

Primary: Default rate of the TB patients with score < 20 by AUDIT. Evaluation of default rate will be obtained from the treatment records of the patients at respective health centres. Treatment outcomes will also be measured in terms of cure rates, treatment failure and death.

Secondary: By comparison of alcohol intake of TB patients' pre and post intervention, the impact of alcohol intervention measures will be evaluated. The study is in progress.

(Contact person: Dr. Beena E Thomas, e.mail: beenathomas@nirt.res.in)

(iii) A community based approach in designing a model TB sensitization programme for self help groups - A study from Tiruvallur district, Tamil Nadu

Primary aim: (i) to design a model sensitization programme on TB for self help groups (SHGs) based on participatory action approach which would facilitate their involvement in the RNTCP

Secondary aims: (i) to promote awareness and on TB among SHGs

(ii) to ascertain the feasibility and acceptability of SHG members in the community for identifying chest symptomatic, referring them for TB investigations and being DOT providers

(iii) to assess the challenges SHGs face (if any) in being involved in the RNTCP programme

Methods: This is an experimental study on a cohort of SHG representatives randomly selected in Tiruvallur district of Tamilnadu.

This study was initiated on May 2012 and was done in phases.

Phase 1: 1a. Situational analysis- In this phase, the number of SHGs in the area, the

number of members in each group, the geographic distribution of SHGs and the profile of SHGs with respect to their socio demographic details were enlisted.

1b. Formative phase- This phase included fixed group discussions (FGDs) and interviews to find out the involvement, of the SHGs, in health programmes and to ascertain their level of awareness of TB, areas which need to be addressed in TB awareness campaigns and their experiences (if any) with TB control programme. This phase also used a community based participatory approach. The intervention manual and the format of the intervention was finalised in this phase.

Phase 2: A cohort of 1400 SHG representatives from 2 blocks, representing various areas in the district will be randomized to an experimental and control group. 761 participants have been recruited so far (429 in the experimental and 332 in the control group). The intervention included interactive sessions, role play, a movie on TB and villupattu covering various topics on TB. These included the cause of TB, spread of TB, prevention of TB, cure of TB, availability of free treatment for TB, need for adherence for TB medication and the role of SHG in the TB control programme.

Outcome Measures

The outcome measures are the number of SHG member involved in TB awareness programme, the number of chest symptomatic identified and referred by SHG member and SHG member who volunteered to be DOTS providers. The study is in progress.

(Contact person: Dr. Beena E Thomas, e.mail: beenathomas@nirt.res.in)

(iv)A study on psycho-social issues facing MDR-TB patients and design appropriate intervention strategies to promote drug adherence

Background: The magnitude of the MDR-TB problem in India is becoming difficult to manage. Studies found MDR-TB levels of about 3% in new cases and 12%-17% in RT cases. The main challenges of dealing with MDR-TB are limited access and poor adherence to treatment as only a small fraction of diagnosed patients is receiving appropriate care. Treatment compliance depends upon the psycho-social behavior of the patient, which is deeply influenced by practices and beliefs ingrained in the culture in which the person is brought up. There is a need therefore, to understand psycho-social and behavioral determinants that would probably lead to better insights into MDR-TB control strategies. Presently, there is dearth of information in India on the psychosocial problems associated with MDR-TB.

Therefore, this study has been planned to assess psychosocial challenges facing MDR-TB patients, and develop an intervention strategy to test the effectiveness of the intervention on adherence as well as their quality of life

Specific aims:

(i) to understand the psychosocial issues facing MDR-TB patients (Depression, stigma, social support)

(ii) to gain insight on the factors that influence treatment adherence and quality of life

(iii) to explore the feasibility and acceptability (effectiveness) of intervention strategies to promote adherence suitable for MDR-TB patients.

The study is approved by the Ethics committee and preliminary work has been initiated.

(Contact person: Dr. E. Thiruvalluvan, email: thiruvalluvane@nirt.res.in)

4

STATISTICAL STUDIES

Department Of Statistics

Overview: The Department of Statistics was created at the formation of the Institute in 1956, to help in plan, design, monitoring and evaluation of clinical trials and related laboratory experiments. The scientists and staff of the department work as collaborative scientists, providing expertise in the statistical methods that form the evidential basis of modern biomedical research. Whether it's uncovering the genetic origins of disease, determining optimal drug therapies for patients, or studying the strategies to reduce health care costs without compromising the quality of patient care - biostatisticians of the department are actively participating in the institute projects.

Apart from actively collaborating and consulting in research projects and initiatives at the Institute, the staff members are pursuing their own research agendas and participating in curriculum development. Expertise in the department includes linear, nonlinear, and longitudinal modeling; clinical trial and experimental design; survival analysis; categorical data analysis; causal inference; HIV and TB disease modeling; computational biology and bioinformatics; machine learning algorithms and data mining; GIS based spatial modeling and Bayesian methodology. The department is offering Ph.D. programmes in the above areas. During the period of review 3 scholars submitted their Ph.D. thesis and 3 scholars submitted synopsis.

The mission of the department is to advance the discipline of Medical Statistics by training researchers in various disciplines in research methods and applications conducting methodological and collaborative interdisciplinary research in the fields of public health and medicine, and by providing expertise to the academic, research and professional committees.

Studies Completed :

(i) Least squares support vector regression for spirometric forced expiratory volume (FEV1) values

Support vector machine (SVM) are one of the recently developed machine learning algorithm under the supervised learning approach, from the statistical learning theory implementing the structural risk minimization (SRM) principle. It maps the data into high dimensional input space and constructs an optimal separating hyperplane in this space. The quality of the solution does not depend directly on the dimensionality of the input space. It has been successful in many real world classification problems like handwritten recognition, object recognition, text categorization, image recognition, classification of gene expression and many more. Regression task in time series prediction, credit scoring has been successfully carried out by SVM. Unlike neural networks, SVMs minimize the estimation error keeping the training error fixed. There are many variants of SVM ever since its existence in the literature. The basic problem in the standard SVM formulation is to solve the QPP, wherein the formulation of least squares support vector machine (LS SVM) focuses on solving a set of linear equations. Thus non-linear pattern recognition is also done by solving a set of linear equation.

Spirometry test is valuable to assess the general respiratory diseases. It is an essential tool in the diagnosis of airway obstruction. We have considered data from 619 (369 males, 250 females) patient's spirometric measurements on FVC and FEV1 and other covariates such

as age, sex, height and weight for empirical comparisons. The prediction of FEV1 values were carried out by linear LS SVM, polynomial LS SVM and RBF LS SVM. We have used LS SVM toolbox to build the model for this classification. It is found that the linear and polynomial models gave similar results in normal and abnormal individuals. Tables 4.1 and 4.2 give the predictions for males. It is found that Linear LS SVM seems to be the better model. The polynomial and linear models performance are similar whereas the RBF model over fits the data. The results of the predictions are presented in tables 4.3 and 4.4 under various models for females. Figs. 4.1 and 4.2 depict comparison of measured and predicated FEV1 values of normal and abnormal female patients.

It is observed that the linear model better fits with respect to the root mean square value in all the cases. A detailed analysis of prediction of FEV1 values for males and females shows the generalization capacity of LS SVM regression and computing FEV1 values through LS SVM regression will enhance the spirometric investigations. In case of incomplete spirometric tests this method may give valuable suggestions and directions (Figs. 4.3 & 4. 4).

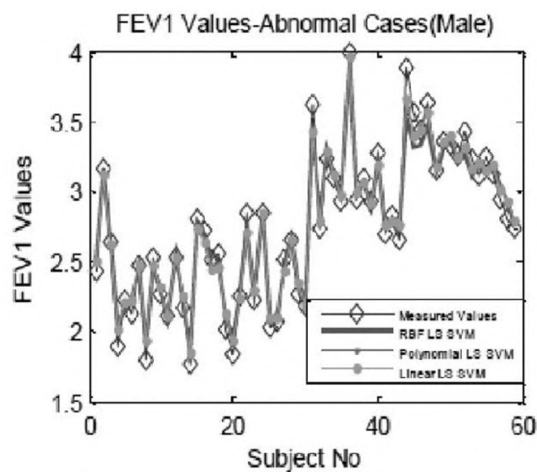

Fig 4.1 FEV_1 values of male abnormal cases

Table 4.1 Normal Cases (Male)

	Measured Values	RBF LS SVM	Polynomial LS SVM	Linear LS SVM
Mean	2.7585	2.7514	2.7622	2.7620
Standard deviation	0.5428	0.4872	0.4963	0.5004
t		0.9705	0.9688	0.9705
Confidence Interval on the mean of the difference		(-0.194, 0.1868)	(-0.1934, 0.1859)	(-0.1821, 0.1534)
RMSE		0.0945	0.0832	0.0817

Table 4.2 Abnormal Cases (Male)

	Measured Values	RBF LS SVM	Polynomial LS SVM	Linear LS SVM
Mean	2.3294	2.3567	2.3465	2.3438
Standard deviation	0.4267	0.4069	0.4327	0.4378
t		0.8657	0.8398	0.8657
Confidence Interval on the mean of the difference		(-0.1821, 0.1534)	(-0.1839, 0.1498)	(-0.1821, 0.1534)
RMSE		0.1238	0.1119	0.1118

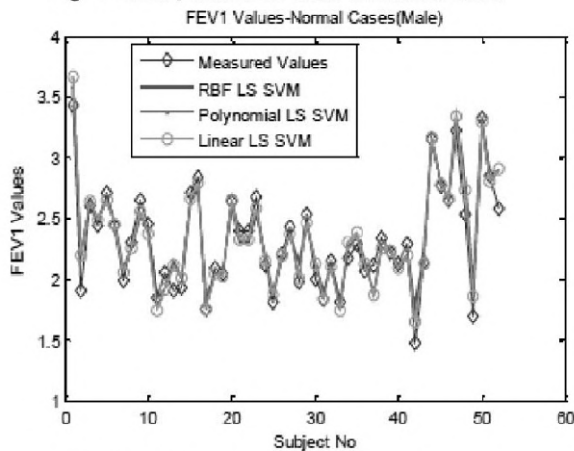

Fig 4.2 FEV_1 values of male normal cases

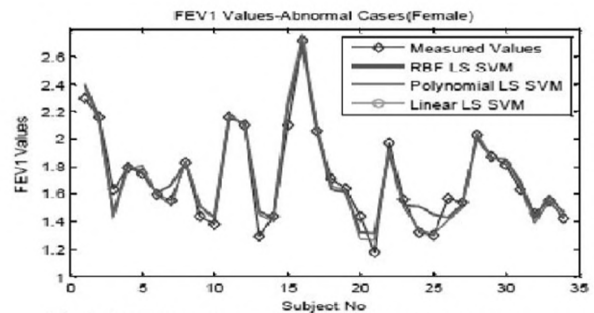

Fig 4.3 FEV_1 values of female abnormal cases

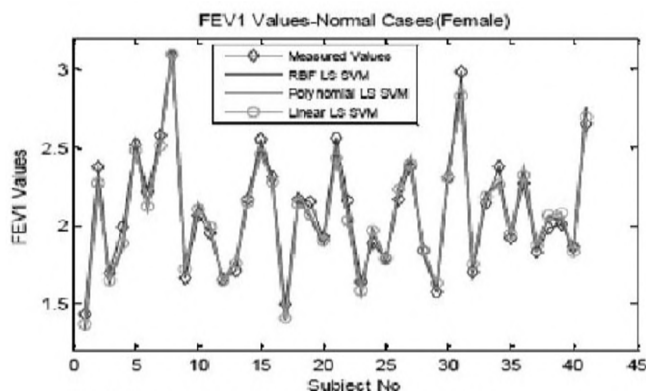

Fig 4.4 FEV_1 values of female normal cases

Table 4.3 Normal Cases (Female)

	Measured Values	RBF LS SVM	Polynomial LS SVM	Linear LS SVM
Mean	2.091	2.0808	2.0735	2.0735
Standard deviation	0.385	0.3894	0.3699	0.3699
t		0.9028	0.8322	0.8321
Confidence Interval on the mean of the difference		(-0.1597, 0.1807)	(-0.1482, 0.1837)	(-0.1482, 0.1837)
RMSE		0.0854	0.0344	0.0344

Table 4.4 Abnormal Cases (Female)

	Measured Values	RBF LS SVM	Polynomial LS SVM	Linear LS SVM
Mean	1.7147	1.7328	1.7152	1.7152
Standard deviation	0.3407	0.3316	0.3620	0.3620
t		0.8249	0.9952	0.9951
Confidence Interval on the mean of the difference		(-0.1809, 0.1447)	(-0.1708, 0.1697)	(-0.1708, 0.1697)
RMSE		0.0802	0.0686	0.0686

(Contact person: Dr. P. Venkatesan, email: venkatesanp@nirt.res.in)

(ii) Bayesian regression approach for modeling response time in clinical trials

Models fitted to survival data will involve parametric or semi-parametric or non-parametric forms for the hazard function. The fitting depends on whether this form is defined as that of a known model, or whether it is completely unknown. In the past three decades, a number of regression-type models have been suggested for the analysis. The Cox PH regression model is a broadly applicable and most widely used method of survival analysis. The other important model in survival analysis is the AFT model. In statistical modeling, heterogeneity may be based on stratification according to factors, regression on covariates or by assuming a probability model of the individual variation. To take such variations into account, frailty models have been recommended. Statistical modeling in Bayesian univariate parametric survival analysis and life testing is too large. Recently parametric models play an important role in Bayesian survival analysis, since many Bayesian analyses in practice are carried out using parametric models. Theoretically, Bayesian techniques offer simple alternatives to statistical inference and all inferences follow from the posterior model. In practice, we can obtain the posterior model with straightforward analytical solutions only in the most rudimentary problems.

Database: For empirical comparison of the different models, we have considered a randomized controlled clinical trial data on TB (TRC, 2007). The aim is to assess the response time to an 8 month treatment regimen consisting of EMB, RMP, INH and PZA thrice weekly for first two months followed by INH and EMB daily for next 6 months. The primary outcome variable is sputum culture conversion time. A total 467 patients were considered for this analysis. Out of these, 90% had favorable response and 10% had not responded or lost which constitute the censored observations. Four important covariates were considered for model comparison namely *age, sex, weight on admission* and *percentage of allocated doses* received by each patient.

Table 4.5 gives the estimated regression coefficients and standard error within parenthesis under different models for Regression and AFT. Table 4.6 shows that the covariate *dosage* is significant for *GE* and *LL* AFT regression models but not significant for other covariates and only *sex* is significant for *W* AFT regression model. When we compare all the AFT regression models, it is found that the *LN* AFT regression model is smaller value of *2LL*, than the other models. Table 4.7 also presents the estimated regression co-efficients Gamma frailty. When we compare all the Frailty regression models it is observed that the value of the *2LL*, of Frailty regression model is smaller than the other models. The *LL* Frailty regression model provides a much better fit compared to the *E, GE, W, G* and *LN* Frailty regression models.

(Contact person: Dr. P. Venkatesan, email: venkatesanp@nirt.res.in)

Table 4.5: Estimated regression of co-efficients under various models

Estimated Regression Coefficients and *SE* Under Various Models for Sputum Culture Conversion Data

	Models	*CoxPH*	*E*	*GE*	*W*	*LL*	*G*	*LN*
	Covariates							
Regression	Sex	−0.193	0.207	0.172*	0.197*	0.139	0.081	0.147
		(0.120)	(0.121)	(0.073)	(0.083)	(0.072)	(0.069)	(0.077)
	Age	−0.036	0.004	0.018	-0.011	0.060	0.061	0.040
		(0.107)	(0.107)	(0.065)	(0.072)	(0.065)	(0.061)	(0.068)
	Weight	0.069	−0.062	−0.044	−0.146	−0.049	−0.015	−0.041
		(0.106)	(0.106)	(0.064)	(0.073)	(0.063)	(0.058)	(0.066)
	Dosage	0.321*	−0.276	−0.198*	−0.190	−0.218*	−0.141	−0.201*
		(0.150)	(0.152)	(0.090)	(0.104)	(0.090)	(0.079)	(0.091)
AFT	Sex	–	0.207	0.172*	0.134*	0.139	0.074	−0.108
			(0.121)	(0.073)	(0.056)	(0.072)	(0.067)	(0.089)
	Age	–	0.004	0.018	−0.007	0.060	0.039	−0.073
			(0.107)	(0.065)	(0.049)	(0.065)	(0.059)	(0.076)
	Weight	–	−0.062	−0.044	−0.031	−0.049	0.028	−0.002
			(0.106)	(0.064)	(0.050)	(0.063)	(0.059)	(0.071)
	Dosage	–	−0.276	−0.198*	−0.129	−0.218*	0.061	0.067
			(0.152)	(0.090)	(0.071)	(0.090)	(0.080)	(0.098)
Frailty	Sex	–	0.207	0.145	0.021	0.139	−0.056*	−0.056*
			(0.121)	(0.076)	(0.016)	(0.072)	(0.026)	(0.026)
	Age	–	0.004	0.041	0.010	0.059	−0.001	−0.001
			(0.107)	(0.068)	(0.014)	(0.065)	(0.023)	(0.023)
	Weight	–	−0.062	−0.041	−0.007	−0.049	0.088	0.008
			(0.106)	(0.066)	(0.013)	(0.063)	(0.023)	(0.023)
	Dosage	–	−0.276	−0.200*	−0.047*	−0.218*	0.018	0.018
			(0.152)	(0.091)	(0.021)	(0.090)	(0.030)	(0.030)
Gamma Frailty	Sex	–	0.129	0.104	0.022	0.041	–	−0.056
			(0.121)	(0.063)	(0.015)	(0.048)		(0.026)
	Age	–	0.015	0.058	0.013	0.013	–	−0.001
			(0.107)	(0.057)	(0.013)	(0.042)		(0.023)
	Weight	–	−0.055	−0.027	−0.005	−0.018	–	0.008
			(0.105)	(0.055)	(0.013)	(0.041)		(0.023)
	Dosage	–	−0.260	−0.163*	−0.044*	−0.095	–	0.018*
			(0.152)	(0.077)	(0.019)	(0.058)		(0.030)

* $p < 0.05$

Table 4.6: Estimated regression co-efficients under various models

Model Selections for Sputum Culture Conversion Data

	Models	*CoxPH*	*E*	*GE*	*W*	*LL*	*G*	*LN*
	Parameter/ Method							
Regression	Scale/Mu	–	0.271	0.573	0.273	0.074	0.511	0.990
	Shape/Sig	–	1.000	3.103	1.468	2.714	1.065	0.654
	(−2LL)	4651	1167	1655	1076	928	890*	938
AFT	Scale/Mu	–	0.271	0.573	0.413	0.382	1.453	0.729
	Shape/Sig	–	–	3.103	1.468	2.714	2.984	0.529
	(−2LL)	–	1824	1655	1733	1585	1603	1326*
Frailty	Scale/Mu	–	0.271	1.577	0.800	0.382	1.027	1.027
	Shape/Sig	–	–	45.469	5.120	2.714	2.703	2.703
	LogSig	–	−8.131	−0.527	−2.090	−8.884	−7.047	−7.047
	(−2LL)	–	1824	1594	1602	1585*	1742	1742
Gamma Frailty	Scale/Mu	–	0.610	1.434	0.442	0.387	–	1.027
	Shape/Sig	–	–	16.627	3.839	3.761	–	2.703
	LogSig	–	−8.765	−1.161	−3.132	−9.964	–	−7.047
	(−2LL)	–	2115	1542*	1553	1966	–	1742
Bayesian: NMC-20000	Scale/Mu	–	0.267	0.551	1.459	2.723	2.832	0.717
	Shape/Sig	–	–	2.943	0.271	0.072	1.374	0.534
	DIC	4656	1834	1667	1745	1597	1615	1338*
Bayesian: NMC-40000	Scale/Mu	–	0.267	0.550	1.459	2.719	2.869	0.716
	Shape/Sig	–	–	2.941	0.271	0.073	1.382	0.534
	DIC	4656	1834	1667	1745	1597	1615	1338*

* Better performance.

Table 4.7: Comparison of various models

Posterior Summaries of Sputum Culture Conversion Data Under Different Bayesian Regression Models

		Sample (NMC = 20000)						Sample (NMC = 40000)					
Models	*Covariates*	*Mean*	*SD*	*MC SE*	*0.25*	*0.50*	*0.75*	*Mean*	*SD*	*MCSE*	*0.25*	*0.50*	*0.75*
CoxPH	Sex	−0.193	0.121	–	−0.275	−0.193	−0.112	−0.192	0.120	–	−0.273	−0.192	−0.111
	Age	−0.035	0.106	–	−0.107	−0.035	0.037	−0.036	0.106	–	−0.108	−0.037	0.035
	Weight	0.069	0.105	–	0.000	0.068	0.140	0.068	0.105	–	−0.002	0.068	0.139
	Dosage	0.286	0.153	–	0.182	0.283	0.388	0.287	0.153	–	0.183	0.284	0.389
E	Sex	0.205	0.117	0.003	0.123	0.207	0.288	0.205	0.120	0.003	0.123	0.207	0.289
	Age	0.006	0.106	0.003	−0.064	0.006	0.078	0.004	0.107	0.002	−0.068	0.005	0.078
	Weight	−0.065	0.107	0.003	−0.136	−0.064	0.008	−0.064	0.107	0.002	−0.136	−0.065	0.009
	Dosage	−0.283	0.152	0.004	−0.384	−0.284	−0.181	−0.284	0.152	0.003	−0.387	−0.283	−0.182
GE	Sex	0.060	0.027	0.001	0.042	0.061	0.078	0.060	0.026	0.001	0.042	0.060	0.077
	Age	0.006*	0.024	0.001	−0.009	0.006	0.022	0.006*	0.024	0.001	−0.009	0.006	0.022
	Weight	−0.015	0.022	0.001	−0.030	−0.015	−0.001	−0.015	0.022	0.001	−0.030	−0.015	0.000
	Dosage	−0.068	0.033	0.001	−0.090	−0.067	−0.044	−0.069	0.033	0.001	−0.090	−0.068	−0.046
W	Sex	0.196	0.083	0.004	0.138	0.195	0.251	0.196	0.082	0.003	0.139	0.196	0.253
	Age	−0.009	0.074	0.003	−0.060	−0.009	0.041	−0.010	0.074	0.002	−0.061	−0.009	0.040
	Weight	−0.042	0.074	0.003	−0.091	−0.041	0.007	−0.042	0.073	0.002	−0.092	−0.041	0.008
	Dosage	−0.197†	0.105	0.009	−0.268	−0.197	−0.128	−0.196	0.106	0.005	−0.268	−0.195	−0.125
LL	Sex	0.386	0.200	0.007	0.258	0.386	0.517	0.378	0.198	0.005	0.247	0.375	0.509
	Age	0.158	0.173	0.006	0.040	0.155	0.271	0.164	0.175	0.004	0.048	0.165	0.282
	Weight	−0.142	0.166	0.005	−0.249	−0.141	−0.027	−0.135	0.168	0.004	−0.245	−0.133	−0.022
	Dosage	−0.610	0.250	0.010	−0.775	−0.607	−0.441	−0.596•	0.247	0.007	−0.755	−0.591	−0.429
G	Sex	0.024	0.025	0.001	0.006	0.024	0.041	0.023	0.025	0.001	0.006	0.024	0.041
	Age	0.014	0.022	0.001	−0.002	0.014	0.029	0.013	0.021	0.001	−0.001	0.013	0.028
	Weight	0.012	0.022	0.001	−0.003	0.012	0.026	0.011	0.021	0.001	−0.003	0.011	0.025
	Dosage	0.023†	0.030	0.002	0.001	0.022	0.044	0.021•	0.029	0.001	0.001	0.021	0.041
LN	Sex	−0.112	0.094	0.003	−0.175	−0.114	−0.050	−0.111	0.093	0.002	−0.172	−0.111	−0.049
	Age	−0.074	0.079	0.002	−0.126	−0.074	−0.021	−0.074	0.078	0.002	−0.125	−0.075	−0.021
	Weight	0.003	0.072	0.003	−0.045	0.002	0.053	0.000	0.072	0.002	−0.048	0.000	0.049
	Dosage	0.058	0.104	0.004	−0.011	0.063	0.128	0.057	0.103	0.002	−0.011	0.062	0.127

* $p < 0.05$, • $p < 0.01$, and † $p < 0.001$

Studies in progress:

(i) Genetic algorithms for disease classification

Genetic algorithms are a part of evolutionary computing, which is a subfield of artificial Intelligence. Biologically, every organism has its own rule to describe how that organism is built up from the tiny building blocks. In the genes of an organism, these rules are encoded and in turn are connected to form long strings called chromosomes. Highly fit chromosomes are selected for reproduction to produce offspring. The offsprings may undergo mutation with less chance. This adaptive search algorithm is used to solve optimization problem with complex search space. To find the classification rule and to select the attributes, in data mining, genetic algorithm is widely applied. The classification is also performed using Naïve Bayes, J48, Naive Bayes, k-Nearest Neibourhood and some hybrid algorithms based on PSO and GA. GA's new fitness function was implemented in Java and tested on diabetes data with Naive Bayes and J48 algorithms.

Knowledge discovered must have the following three things, predictive accuracy, comprehensive, and the result should be used to predict something or to compare two or more results. Data mining is a particular case of KDD. Data mining includes, Classification, Clustering, Regression, relation among attributes, description of data, dependency modeling, and sequence analysis. Among them Classification was carried out by statistical methods and machine learning for many years. Genetic algorithms help to get classification rules, such that it gives good prediction accuracy. The rules is of the form "if Antecedent then consequent", i.e., if x then y, where x is the antecedent of the rule and y is the consequent of the rule, that is the predicted class. Using the confusion matrix, we get t*rue positive (tp),* f*alse positive (fp),* t*rue negative (tn),* f*alse negative (fn)*. In genetic algorithm a classification rule is represented by chromosomes and the gene represent the attribute's value. The dataset was usually divided into training and test set. By coding the instances of the training set, the initial population of rules is obtained. The genetic algorithm was implemented in Java and tested on diabetic data using WEKA and the classic algorithms NaïveBayes and J48 also tested on the same data (Tables 4.8-4.10).

Table 4.8: Naive bayes classifier

Class	TP Rate	FP Rate	Precision	Recall	F-Measure	ROC Area
Tested_Negative	0.842	0.384	0.803	0.842	0.822	0.825
Tested_Positive	0.616	0.158	0.676	0.616	0.645	0.825
Weighted Avg.	0.763	0.305	0.759	0.763	0.76	0.825

Table 4.9: J48 classifier

Class	TP Rate	FP Rate	Precision	Recall	F-Measure	ROC Area
Tested_Negative	0.936	0.336	0.839	0.936	0.885	0.888
Tested_Positive	0.664	0.064	0.848	0.664	0.745	0.888
Weighted Avg.	0.841	0.241	0.842	0.841	0.836	0.888

Table 4.10: Genetic algorithm classifier

Class	TP Rate	FP Rate	Precision	Recall	F-Measure	ROC Area
Tested_negative	0.849	0.379	0.808	0.849	0.828	0.821
Tested_positive	0.621	0.151	0.686	0.621	0.652	0.821
Weighted Avg.	0.77	0.3	0.766	0.77	0.767	0.821

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(ii) Classification of TB microarray data using support vector machine

Microarray technology is used to monitor the expression levels of genes on a genomic scale. It increases the possibility of disease classification and disease diagnosis at the gene expression evel. Microarray experiments provide a wealth of information, however extensive data mining is required to identify the patterns that characterize the underlying mechanisms of action. In recent years, the processing of high-throughput biological data has evolved into a highly interdisciplinary field and a large number of machine learning algorithms have been proposed to automate difficult tasks that of medical diagnosis from gene expression profiles. SVM is a supervised machine learning technique used for data classification. It performs classification by constructing an optimal hyper plane which separates the data into two classes. SVM was developed from statistical learning theory. A SVM optimally separates two different classes of data by a hyper plane. The points lying on the boundaries are called support vectors and the middle of the margin is the optimal separating hyper plane SVM classifiers have been applied to multi-spectral and hyper spectral data. SVM classifiers represent a promising nonparametric classification method for identifying and differentiating different land cover types. A modified version of SVM, the LS SVM, employs a set of mapping functions to map the input data into the reproducing kernel Hilbert space, where the mapping function is implicitly defined by a kernel function. SVM has shown promise in a variety of biological classification tasks, including gene expression micro arrays.

SVM: The three important steps of support vector machine are: (i) Project the data from the known classes into suitable high dimensional space, (ii) Identify a hyper plane that separates the two classes and (iii) The class of the new individual is determined by the side of the hyper plane on the sample lines.When the points are separated by a nonlinear region, the SVM handles this by using a kernel function in order to map the data into a different space, when the hyper plane can be used to do the separation. The hyper plane in the higher dimensional space is defined as the set of points whose inner product with a vector in that space is constant. The effectiveness of SVM depends on the selection of kernel, the kernel's parameters, and soft margin parameter. Typically, each combination of parameter choices is checked using cross validation and the parameters with best cross validation accuracy is picked. The final model which is used for testing and for classifying new data, is then trained on the whole, training set using the selected parameters.

Data sets: We have considered the gene expression data of mycobacterium tuberculosis from Stanford Microarray database. The database No. GSD1552 is based on parent platformGPL2787 with reference series GSE3201. It is a double channel microarray data of *M. tuberculosis*. Chanel 1 contains H37Rv genomic DNA control which is labeled with cy3, and channel 2 contains CDC 1551 genomic DNA which is labeled with cy5. We have used normalized log ratio values for each gene.

Results and conclusion: We have used statistical analysis software MATLAB version 7.10.0 for performing the classification using sequential minimal optimization (SMO) algorithm which supports SVM. First, SVM is trained using kernel function. Before training the columns of the input, data matrix training are shifted to zero mean and scaled to unit variance. After training, SVM produces a structure which contains the information about the trained classifier. Once the training is completed the classification accuracy is evaluated using the training set as well as testing set by 10 fold cross validation. The classification performance is evaluated by correct rate cp =0.98, which shows 98% classification accuracy. It is noted that the number of genes in the gene list and the number of cases

(samples) substantially influence classification success. The SVM classifier shows higher classification efficiency and reveals excellent prediction and classification performance in gene expression microarray analysis. Ther study is in progress.

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(iii) Bayesian kriging of TB prevalence

Kriging is a technique used in the analysis of spatial data. The data from the measured location can be used to estimate the variable at the location where it had not been measured. This extrapolation from measured location to unmeasured location is called kriging. Measurements of variable at a set of points in a region are used to extrapolate points in the region where the variable was not measured outside the region that we believe will behave similarly. In both cases, we will need to first fit a variogram model to our data. The three major functions used in spatial statistics for describing the spatial correlation of observations are the correlogram, the covariance, and the semi-variogram. The last is also more simply called the variogram. The variogram is the key function in spatial statistics as it is used to fit a model of the spatial correlation of the data.

Observations made at different locations may not be independent and highly correlated. The spatial autocorrelation value can be positive or negative. Positive spatial autocorrelation occurs when similar values occur near one another. Negative spatial autocorrelation occurs when dissimilar values occur near one another. If there is any systematic pattern in the spatial distribution, it is said to be spatially autocorrelated. One approach is to define disease in a ward to be close to one another, and then determine, whether pattern may have similar characteristics. Once the spatial correlation structure of a variable has been identified, the data from the measured locations can be used to estimate the spatial dependence based on location is significant or not. The data from the measured location can be used to estimate the variable at the location where it had not been measured. This extrapolation from measured location to unmeasured location is called kriging. This method of Prediction is based on the assumption that covariance between points is entirely a function of distance between them as modeled by means of the variogram. Further, it is assumed that the underlying mean of the quantity being predicted is constant.

The datas pertaining to the patients registered during 2004 to 2006 for an ongoing trial were considered for this study. Chennai had 155 wards and for each ward the total number of TB cases recorded between 2004 and 2006 were identified. For variogram analysis, 28 wards of Chennai district were selected for our study for which the locations of 72 cases were geographically marked through their co-ordinates in the Chennai map. Kriging analysis was carried out using SAS software by dividing the whole area into some 100 by 100 grid matrix and prediction was calculated using ordinary kriging method. For Bayesian kriging, WinBUGS software was used for prediction of certain locations based on available information about other location. The spatial prediction permits spatial interpolation and prediction in WinBUGS. The spatial.exp function allows the fitting of a fully parameterized covariance function within a multivariate normal distributional model. Spatial.unipred provides a method of predicting values of the fitted surface at unsampled locations (Fig. 4.5). Table 4.11 shows the observed and predicted values of the SMR in Chennai wards.

Table 4.11: Kriging Estimates

Wards	SMR	Ordinary Kriging		Bayesian Kriging	
		Prediction	Std Error	Prediction	Std Error
Ward1	0.469	0.359	0.11	0.431	0.038
Ward 2	1.611	1.181	0.43	1.591	0.02
Ward 3	0.628	0.428	0.2	0.614	0.014
Ward 4	0.646	0.87	0.224	0.635	0.011
Ward 5	0.352	0.232	0.12	0.362	0.01
Ward 6	0.609	0.94	0.331	0.611	0.002
Ward 7	2.492	0.942	1.55	2.123	0.369
Ward 8	0.858	0.488	0.37	0.812	0.046
Ward 9	0.701	0.211	0.49	0.712	0.011
Ward 10	1.01	1.31	0.3	1.11	0.1

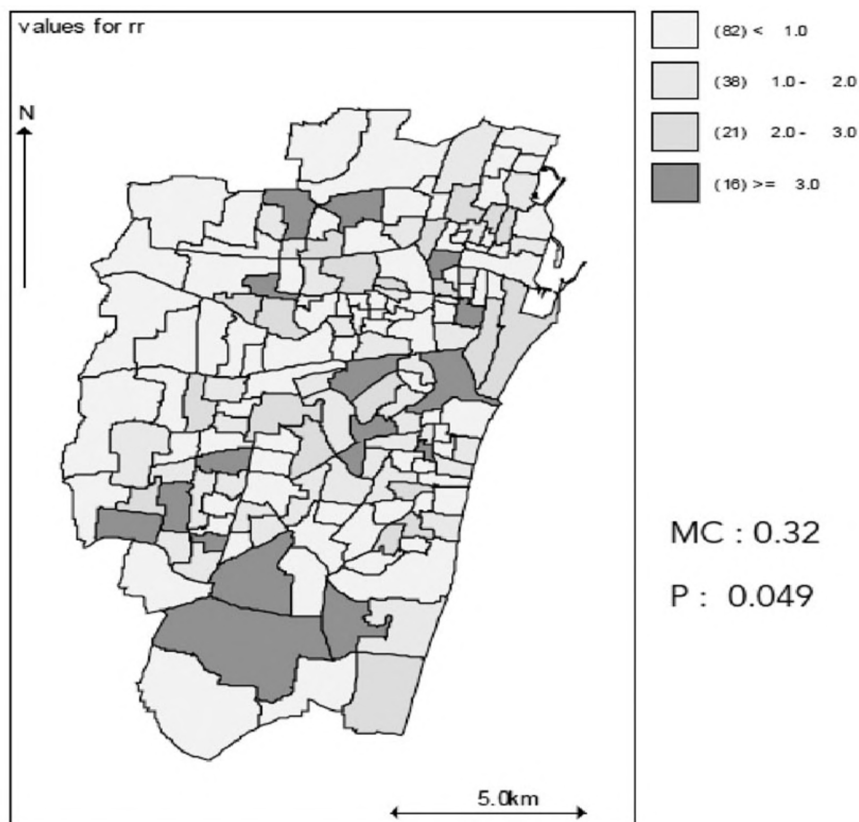

Fig. 4.5: Measure of spatial autocorrelation for Chennai wards

Bayesian Kriging is more appropriate for spatial data modeling. The Moran's spatial autocorrelation of Chennai city is 0.32 which attains border line significant.

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5
EPIDEMIOLOGICAL STUDIES
Department of Epidemiology

Overview : Epidemiology started its work since 1968 BCG trial in TB in Chingelput, district. This area utilized as the population laboratory for the study of the epidemiology of TB. After the BCG Trial, several disease prevalence surveys were undertaken by NIRT Epidemiology unit. The RNTCP was introduced in the BCG trial area in 1999. Currently the disease survey and tuberculin survey are being carried out in the BCG trial area to study the impact of DOTS strategy (RNTCP) on the epidemiology of TB. A case control study was done in 1998 to study the association between Tobacco smoking and PTB. In the ongoing disease survey, the information on exposure to risk factors like tobacco smoking, alcohol consumption are being collected to study the association between these behavioral risk factors with PTB. The epidemiology unit of TRC has also collaborated with National Tuberculosis Institute, Bangalore, in conducting a nation wide sample survey (tuberculin survey). Studies on mortality among cohorts of tuberculosis patients from Chennai Corporation and Thiruvallur district were completed. Mortality surveys were completed in Andhra Pradesh and Orissa.

Study completed:

(i) Estimation of prevalence of PTB in Chennai city

As it was anticipated that the prevalence of pulmonary tuberculosis is high in urban areas, this study was carried out in the Chennai metropolitan city. The first survey was initiated in July 2010 and completed by October 2011. For this survey, the sample size was calculated assuming a prevalence of 400/100000 population, precision of 25%, a design effect of 1.3 and 25% missing of eligible cases. Accordingly the sample size was estimated to be 26,529. The sample size will be distributed among 50 clusters (here, Wards) with a cluster size of 531 (IJTLD 2008, 1003-1008). So approximately 600 adult population (>15 years) were enumerated in each ward giving due coverage to both slum and non-slum areas (ie from Slum-200, from Non-Slum 400). Attempts were made to screen all Individuals for TB for chest symptoms (using a questionnaire) and chest radiography using MMR (mass miniature radiograph).

A second survey was planned immediately after the first survey, to measure if there is any change in the TB prevalence. The sampling design was the same as that of the first survey, however due to administrative issues X-ray screening could not be done in 6 wards that were surveyed initially. However, for the remaining 44 wards, both X-ray and symptom screening were carried out. The second survey was carried out during October 2011-October 2012.

Results:

The consolidated details of coverage in the 1st and II round of surveys is shown in table 5.1. Those wards in which the X-ray screening was not done, were not included

Table 5.1: Details of coverage under the 2 prevalence surveys

Details	Coverage details	
	Survey I (50 wards)	Survey II (44 wards)
Total enumerated	29998	266361
Number screened for chest symptoms	27800 (93%)	24499 (92%)
Number screened for X-ray	27158 (91%)	23820 (89%)
Number eligible for sputum (based on X-ray or chest symptoms)	2786	2743
Number of individuals who gave sputum samples	2538 (91%)	2497 (91%)
Number of smear and/or culture positives	90	74

A total of 29,998 and 26,631 individuals were found eligible and enumerated in the 1st and II round of surveys. Coverage for both symptom and X-ray screening was almost 90% in both surveys. Of those eligible for sputum collection, 91% provided with sputum samples. Age and gender wise prevalences of TB adjusted for non coverage under X-ray screening and sputum collected in I and II surveys are given in tables 5.2 and 5.3 respectively.

Table 5.2: Age and gender wise TB prevalence in I survey

Age groups	Eligible population	Coverage for Xray	Eligible for sputum	Coverage for sputum collection	Smear and /or culture positve	Positives after correcting for non coverage of X ray	Prevalence per 1000
Among males							
5-24	3587	92.14	235	88.09	5	6	1.72
25-34	3702	84.68	266	89.47	8	11	2.85
35-44	3280	84.76	359	91.92	16	21	6.26
45-54	2390	85.31	338	87.87	21	28	11.72
55-64	1468	86.72	284	91.20	17	21	14.64
>=65	1027	89.97	224	91.07	7	9	8.32
All Ages	15454	87.07	1706	89.98	74	94	6.11
Among females							
15-24	3009	92.79	152	90.79	1	1	0.39
25-34	3328	93.66	202	92.57	1	1	0.35
35-44	3310	94.50	218	94.04	4	5	1.36
45-54	2291	94.94	211	91.00	3	3	1.52
55-64	1568	95.03	157	94.90	5	6	3.54
>=65	1038	96.15	140	94.29	2	2	2.13
All Ages	14544	94.20	1080	92.87	16	18	1.26
Among total surveyed							
15-24	6596	92.43	387	89.15	6	7	1.10
25-34	7030	88.93	468	90.81	9	11	1.59
35-44	6590	89.65	577	92.72	20	24	3.65
45-54	4681	90.02	549	89.07	24	30	6.39
55-64	3036	91.01	441	92.52	22	26	8.61
>=65	2065	93.08	364	92.31	9	10	5.07
All Ages	29998	90.53	2786	91.10	90	109	3.64

Table 5.3: Age and gender wise TB prevalence in II survey

Age groups	Eligible population	Coverage for Xray	Eligible for sputum	Coverage for sputum collection	Smear and /or culture positve	Positives after adjusting for non coverage of X ray	Prevalence per 1000
Among males							
15-24	2771	91.81	162	93.21	4	5	1.69
25-34	3038	84.92	234	89.32	4	5	1.74
35-44	2843	83.71	324	91.05	13	17	6.00
45-54	2145	82.52	339	89.38	15	20	9.48
55-64	1297	86.82	275	90.91	12	15	11.72
>=65	929	91.60	224	92.41	10	12	12.72
All Ages	13023	86.39	1558	90.82	58	74	5.68
Among females							
15-24	2536	92.43	129	96.90	1	1	0.44
25-34	3155	93.85	207	88.89	1	1	0.38
35-44	2913	94.54	238	92.44	2	2	0.79
45-54	2174	94.62	205	93.66	4	5	2.08
55-64	1457	95.47	172	88.37	3	4	2.44
>=65	1103	96.28	234	89.32	5	6	5.27
All Ages	13338	94.23	1185	91.31	16	19	1.39
Among all those surveyed							
15-24	5307	92.10	291	94.85	5	6	1.08
25-34	6193	89.47	441	89.12	5	6	1.01
35-44	5756	89.19	562	91.64	15	18	3.19
45-54	4319	88.61	544	90.99	19	24	5.46
55-64	2754	91.39	447	89.93	15	18	6.63
>=65	2032	94.14	458	90.83	15	18	8.63
All Ages	26361	90.36	2743	91.03	74	90	3.41

It may be seen from the Tables 5.2 and 5.3 that the prevalence of TB increased with age and it was significantly lower among females when compared to males (P value <0.001) in both surveys. Further it was seen that the prevalence of TB between the I (3.64 per 1000 population, 95% CIs (2.95-4.31) and II (3.41 per 1000 population, 95% CIs (2.71 4.11)) surveys did not vary significantly (chi square=0.19, df=1, P value=0.66).

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6

INTERNATIONAL CENTRE OF EXCELLENCE IN RESEARCH

Studies Completed :

(i) Host immune responses in lymphatic filariasis

(ii) Altered spontaneous and antigen - specific frequencies of Th1, Th2, Th17 and T-cells expressing IL-10 family cytokines in filarial lymphedema

Background: Lymphatic filariasis can be associated with development of serious pathology in the form of lymphedema, hydrocele, and elephantiasis in a subset of infected patients. Dysregulated host inflammatory responses leading to systemic immune activation are thought to play a central role in filarial disease pathogenesis. Lymphatic filarial disease is known to be associated with elevated Th1 and normal or diminished Th2 responses to parasite-specific antigens. The role of Th17 cells and T-cells expressing the IL-10 family of cytokines, however, has not been well-defined nor has the contribution of $CD8^+$ T-cells to this cytokine response.

Aim: (i) to study the role of $CD4^+$ and $CD8^+$ Th1, Th2 and Th17 cell subsets as well as T-cells expressing IL-10 family of cytokines in the development of lymphatic pathology

Methods : We examined the frequency of these cells in individuals with filarial lymphedema (chronic pathology) directly ex vivo and in response to parasite or non-parasite antigens by intracellular cytokine multi-parameter flow cytometry; these frequencies were compared to those in clinically asymptomatic filarial-infected individuals (INF).

Results : CP individuals exhibited a significant increase in the frequency of $CD4^+$ T-cells expressing IL-2, TNF- , IFN , IL-17 and IL-22 at baseline and in response to filarial antigens compared to INF individuals (Fig. 6.1). In contrast, these same individuals exhibited a significant decrease in the frequency of $CD4^+$ T-cells expressing IL-4, IL-5, IL-9, IL-13 and IL-21. In addition, CP individuals displayed a significant decrease in the frequency of $CD4^+$ T-cells expressing IL-10, IL-19 and IL-24 but not IL-26. These differential frequencies between the 2 patient groups were also observed in $CD8^+$ T-cells, albeit to a much less extent.

Conclusion: Our findings suggest that alterations in the frequencies of $CD4^+$ and $CD8^+$ Th1, Th2, Th17 cells and T-cells expressing IL-10 family of cytokines are a characteristic feature underlying the pathogenesis of filarial lymphedema.

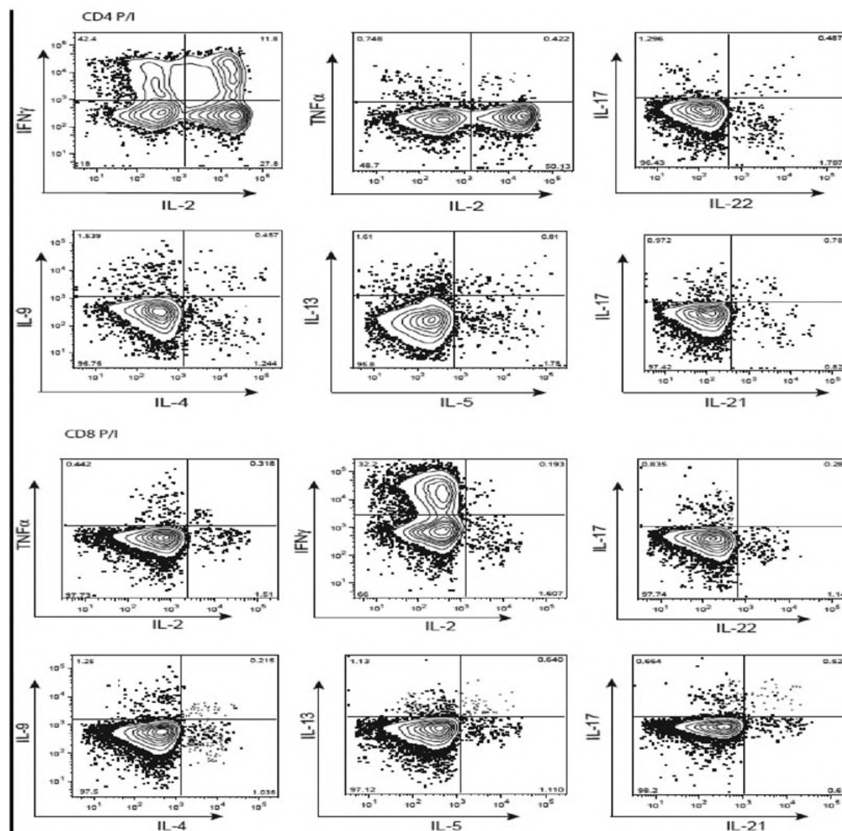

Fig. 6.1: Production of cytokines in filarial pathology

A representative flow plot depicting the antigen and mitogen induced production of Th1 (IL-2, TNF- and IFN-), Th2 (IL-4, IL-5 and IL-13) and Th17 (IL-17, IL-21 and IL-22) cytokines in a CP individual.

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(ii) Parasite antigen-specific, IL-4-, TGF - and IL-1- dependent expansion of Th9 cells is associated with clinical pathology in human lymphatic filariasis

Background: Th9 cells are a subset of CD4$^+$ T-cells, shown to be important in allergy, autoimmunity and anti-tumor responses. However, their role in human infectious diseases has not been explored in detail.

Aim: (i) to elucidate the role of Th9 cells in filarial pathology

Methods: We studied the expression patterns of Th2 and Th9 cells in normal individuals, individuals with pathology and asymptomatic, filarial-infected individuals by multi-parameter flow cytometry and ELISA. We also did in vitro assays to determine the mechanism of regulation of these cell populations.

Results: We identified a population of IL-9 and IL-10 co-expressing cells (lacking IL-4 expression) in normal individuals that respond to antigenic and mitogenic stimulation but are distinct from IL-9$^+$ Th2 cells (Fig. 6.2). We also demonstrate that these Th9 cells exhibit antigen specific expansion in a chronic helminth infection (lymphatic filariasis). Comparison of Th9 responses reveals that individuals with pathology associated with filarial infection exhibit significantly expanded frequencies of filarial antigen induced Th9 cells but not of IL9$^+$Th2 cells in comparison to filarial-infected individuals without associated disease (Fig. 6.2). Moreover, the per cell production of IL-9 is significantly higher in Th9 cells compared to IL9$^+$Th2 cells, indicating that the Th9 cells are the predominant CD4$^+$ T-

cell subset producing IL-9 in the context of human infection. This expansion was reflected in elevated antigen stimulated IL-9 cytokine levels in whole blood culture supernatants. Finally, the frequencies of Th9 cells correlated positively with the severity of lymphedema (and presumed inflammation) in filarial diseased individuals. This expansion of Th9 cells was dependent on IL-4, TGF and IL-1 *in vitro.*

Conclusion: We have therefore identified an important human $CD4^+$ T-cell subpopulation co expressing IL-9 and IL-10 but not IL-4, whose expansion is associated with disease in chronic lymphatic filariasis and could potentially play an important role in the pathogenesis of other inflammatory disorders.

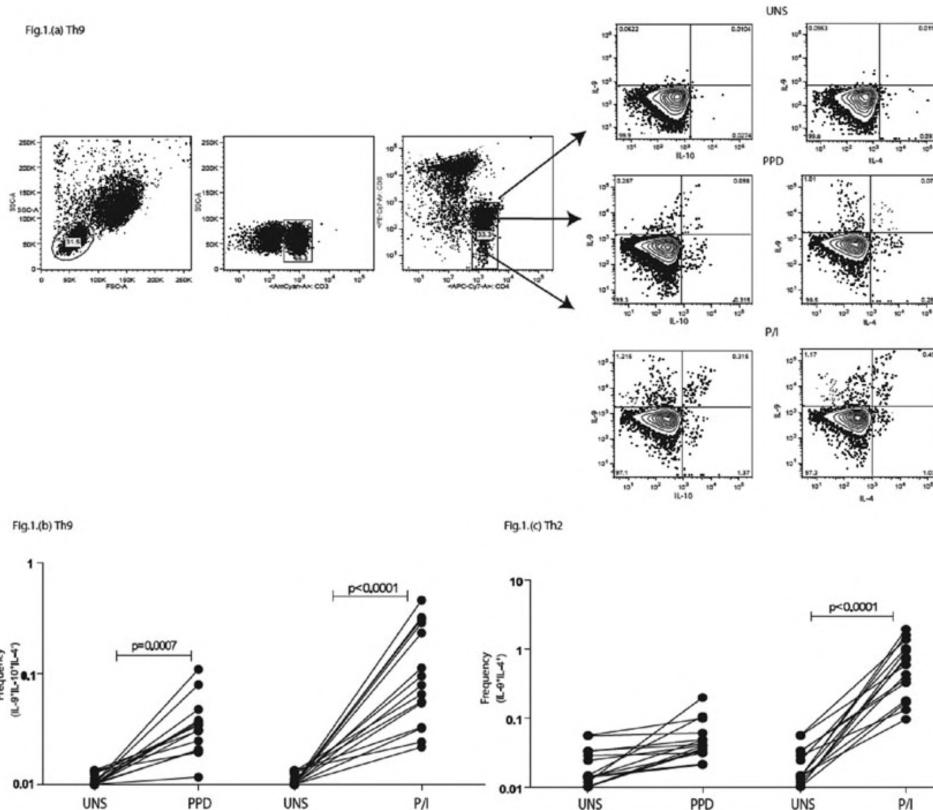

Fig. 6.2: Identification of classical Th9 cells and their expansion in normal individuals

(A) A representative flow plot depicting the gating strategy and baseline as well as PPD and PMA/ ionomycin stimulated population of $CD4^+$ T-cells expressing IL-9, IL-4 and IL-10 in a normal individual

(B) Baseline, PPD and PMA/ ionomycin induced frequencies of $CD4^+$ T-cells expressing IL-9 and IL-10 but not IL-4 (classical Th9 cells) in normal individuals (n=15)

(C) Baseline, PPD and PMA/ ionomycin induced frequencies of $CD4^+$ T-cells expressing IL-9 and IL-4 (Th2 cells) in normal individuals (n=15). The data are represented as frequencies of $CD4^+$ T-cells and each line represents a single individual

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(iii) Parasite-antigen driven expansion of $IL\text{-}5^-$ and $IL\text{-}5^+$ Th2 human subpopulations in lymphatic filariasis and their differential dependence on IL-10 and TGF for expansion

Background: Two different Th2 subsets have been defined recently on the basis of IL-5 expression an $IL\text{-}5^+$, Th2 subset and an $IL\text{-}5^-$, Th2 subset in the setting of allergy. However, the role of these newly described $CD4^+$ T-cells subpopulations has not been explored in other contexts.

Aim and Methods :

(I) to study the role of the Th2 subpopulation in a chronic, tissue invasive parasitic infection (lymphatic filariasis), we examined the frequency of $IL\text{-}5^{+}IL\text{-}4^{+}IL\text{-}13^{+}$ $CD4^{+}$ T cells and $IL\text{-}5^{-}$ IL-4 $IL13^{+}$ $CD4^{+}$ T-cells in asymptomatic, infected individuals (INF) and compared them to frequencies (F_o) in filarial-uninfected (UN) individuals and to those with filarial lymphedema (CP)

Results: INF individuals exhibited a significant increase in the spontaneously expressed and antigen-induced F_o of both Th2 subpopulations compared to the UN and CP. Interestingly, there was a positive correlation between the F_o of $IL\text{-}5^{+}$Th2 cells and the absolute eosinophil and neutrophil counts; in addition there was a positive correlation between the frequency of the $CD4^{+}IL\text{-}5^{-}$ Th2 subpopulation and the levels of parasite antigen specific IgE and IgG4 in INF individuals. Moreover, blockade of IL-10 and/or TGFb demonstrated that each of these 2 regulatory cytokines exert opposite effects on the different Th2 subsets. Finally, in those INF individuals cured of infection by anti-filarial therapy, there was a significantly decreased F_o of both Th2 subsets.

Conclusion: Our findings suggest that both $IL\text{-}5^{+}$ and $IL\text{-}5^{-}$Th2 cells play an important role in the regulation of immune responses in filarial infection and that these two Th2 subpopulations may be regulated by different cytokine-receptor mediated processes.

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II. Immunology of helminth-TB co-infections

(i) Modulation of mycobacterial-specific Th1 and Th17 cells in latent TB by coincidenthookworm infection

Background: Hookworm infections and TB are co-endemic in many parts of the world. It has been suggested that infection with helminth parasites could suppress the predominant Th1 (IFN--mediated) response needed to control *M. tuberculosis* infection and enhance susceptibility to infection and/or disease.

Aim and Methods:

(i) to determine the role of coincident hookworm infection on responses at steady state and on Mtb specificimmune responses in LTB, we examined the cellular responses in individuals with latent TB with or without concomitant hookworm infection by flow cytometry and ELISA

Results: By analyzing the expression of Th1, Th2 and Th17 subsets of $CD4^{+}$ T cells, we were able to demonstrate that the presence of coincident hookworm infection significantly diminished both spontaneously expressed and *M. tuberculosis* specific mono and dual functional Th1 and Th17 cells. Hookworm infection, in contrast, was associated with expanded frequencies of mono and dual functional Th2 cells at both steady state and upon antigen stimulation. This differential induction of $CD4^{+}$ T-cell subsets was abrogated upon mitogen stimulation. In addition, coincident hookworm infection was associated with increased adaptive T regulatory (aTreg) cells but not natural regulatory T- cells (nTregs) in latent TB. Finally, the $CD4^{+}$ T-cell cytokine expression pattern was also associated with alterations in the systemic levels of Th1 and Th2 cytokines (Fig. 6.3).

Conclusion: Thus, coincident hookworm infection exerts a profound inhibitory effect on protective Th1 and Th17 responses in latent tuberculosis and may predispose toward the development of active TB in humans.

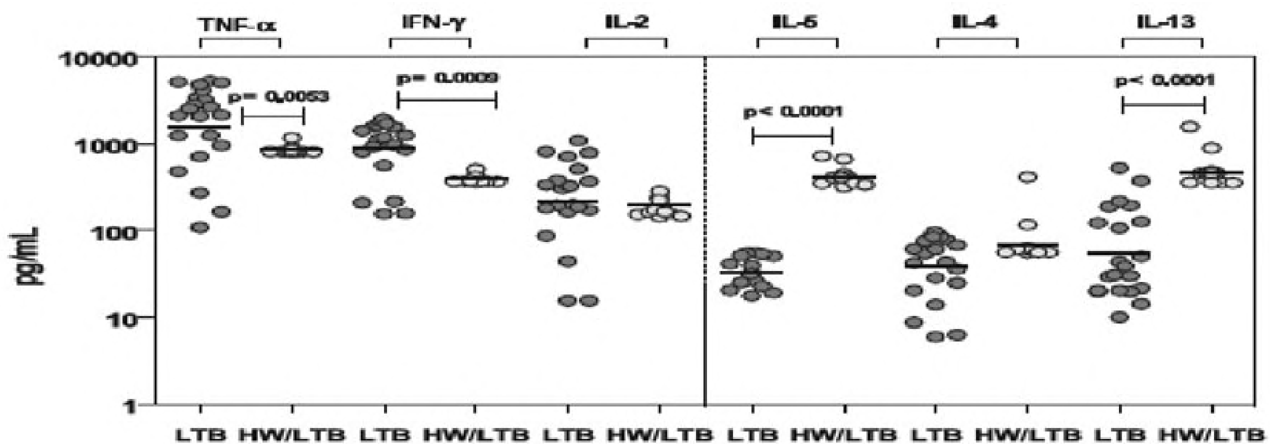

Fig. 6.3: Hookworm infection is associated with alterations in the plasma levels of Th1 and Th2 cytokines in latent TB

The plasma levels of Th1 - IFN-, IL-2, TNF- and Th2 IL-4, IL-5, IL-13 cytokines were measured by ELISA in latent TB infected individuals with (HW/LTB; n=21) or without (LTB; n=21) concomitant hookworm infection. The results are shown as scatterplots with each circle representing a single individual.

(Contact person: Dr. S. Subash Babu, email: sbabu@nirt.res.in)

II. Immunology of TB and its co-morbidities

(i) Plasma heme oxygenase-1 is a surrogate marker of active disease and sputum positivity in human TB

Background: TB is associated with oxidative stress as well as the induction of host anti-oxidants to counteract this response. Recently, the enzyme heme oxygenase-1 (HO-1) has been implicated as a critical promoter of cytoprotection in diverse disease models including mycobacterial infection. Nevertheless, the pattern of expression of HO-1 in human TB has not been previously studied.

Aim: (i) to examine the expression of HO-1 in *M. tuberculosis*-exposed and -infected individuals and test its ability to distinguish active, latent and successfully treated TB and healthy controls

Methods: Cross-sectional and longitudinal analyses of plasma levels of HO-1, acute phase proteins and pro-inflammatory cytokines were performed in samples from individuals with active PTB (n=97); extra-pulmonary TB (ETB) (n=35); latent TB infection (n=39) and healthy controls (n=40) as part of a prospective cohort study in a population highly endemic for TB from south India.

Results: Systemic levels of HO-1 were dramatically increased in individuals with active pulmonary and extra-PTB and particularly those with bilateral lung lesions and elevated bacillary loads in the sputum. HO-1 levels effectively discriminated active from latent TB and healthy controls. Moreover, there was a marked reduction in HO-1 levels in active TB cases following anti-tuberculous therapy but not in those with treatment failure. In addition, plasma HO-1 was a significantly better discriminator of disease outcome in TB when compared to two other acute phase reactants, C-reactive protein and serum Amyloid Protein-A (Fig. 6.4).

Conclusion: These findings establish HO-1 as a sensitive and accurate biomarker for active PTB.

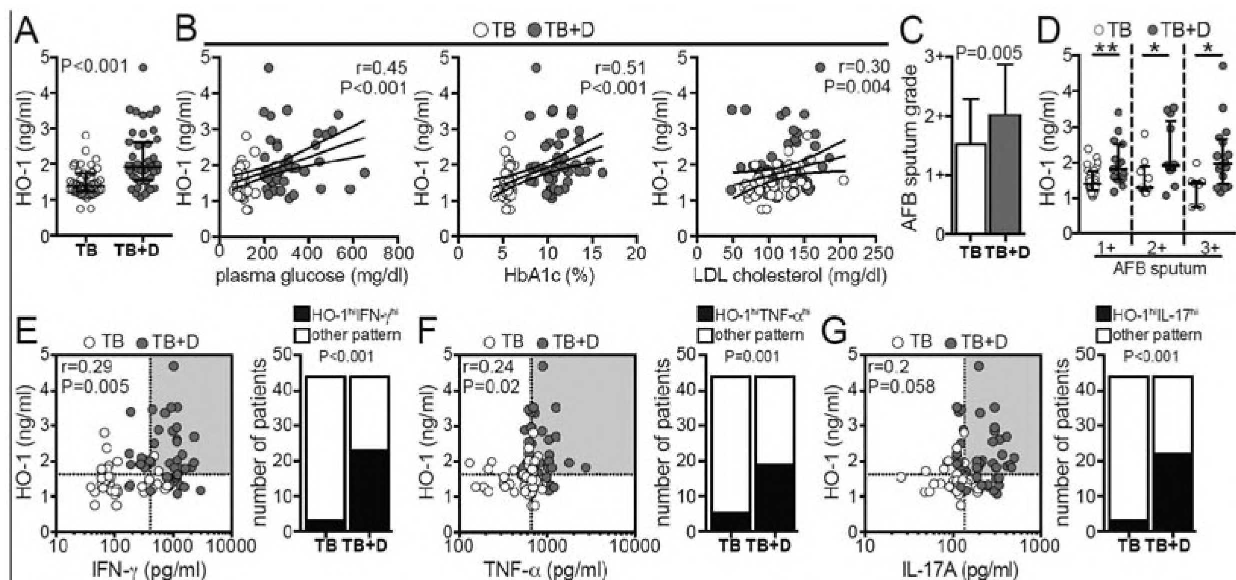

Fig. 6.4: HO-1 is associated with disease activity and sputum positivity in TB

(A) HO-1 levels were evaluated in plasma samples from 97 patients with active PTB, 35 patients with active extra pulmonary tuberculosis (EPTB), 39 individuals with latent *M. tuberculosis* infection (LTBI) and 40 healthy donors (HD). The samples were collected prior to initiation of ATT. Kruskal-Wallis test and Dunn's multiple comparisons were used to evaluate statistical differences between the groups.

(B) HO-1 levels were compared between 40 PTB patients with a diagnosis of unilateral lung lesions and 37 patients with bilateral lesions identified by chest radiography.

(C) PTB cases were classified according to the presence or absence of the acid fast staining bacilli (AFB) in sputum samples. In (B) and (C), the Mann Whitney test was used for statistical comparisons.

(D) PTB cases were stratified according to the quantitative bacillary sputum grade determined by AFB staining. Data was analyzed using the Kruskal-Wallis test with linear trend post-test (left panel) or by Spearman correlation (right panel). In (A), (B), (C) and (D), bars represent median values.

(E) Comparison of HO-1 levels pre and post ATT in a subset of patients with PTB for which plasma samples were available (n=33). Data from 5 patients that failed treatment are also shown. The Wilcoxon matched pairs test was used to evaluate the significance of the effect of ATT on HO-1 values. *P<0.05; **P<0.01; ***P<0.001.

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(ii) Type 2 diabetes mellitus coincident with PTB is associated with heightened systemic Type 1, Type 17 and other pro inflammatory cytokines

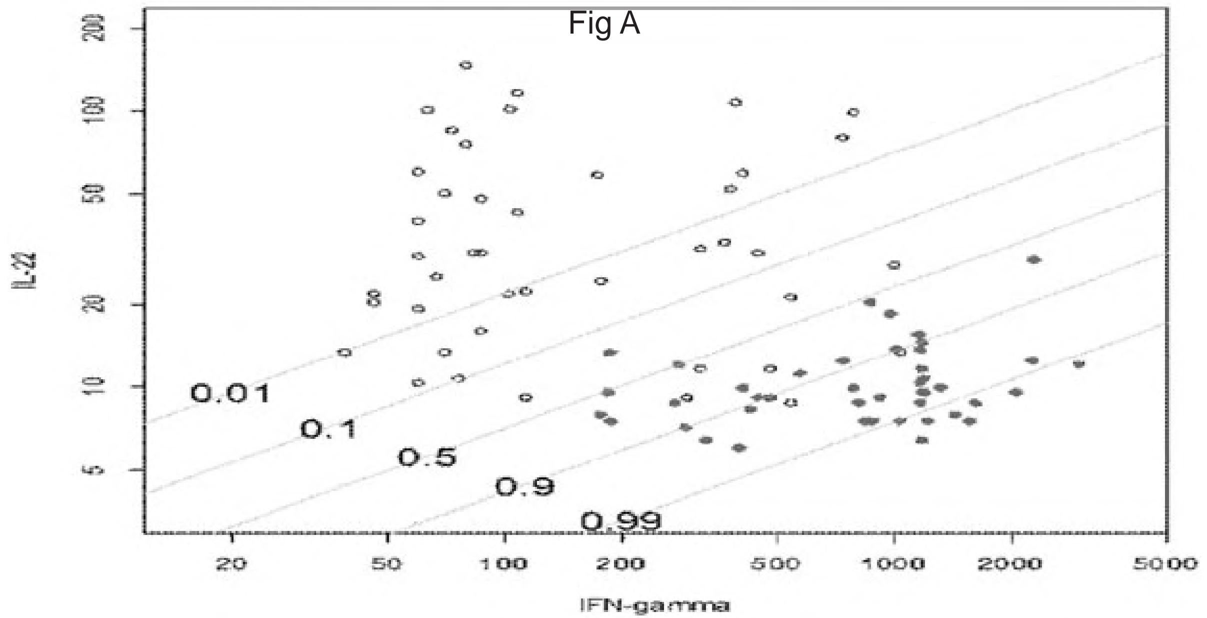
Background: Type 2 diabetes mellitus is a major risk factor for the development of active TB, although the biological basis underlying this susceptibility remains poorly characterized.

Aim and Methods:

(i) to identify the influence of coincident diabetes mellitus on cytokine levels in PTB, we examined circulating levels of a panel of cytokines and chemokines in the plasma of individuals with TB with diabetes and compared them with those without diabetes

Results: TB with diabetes is characterized by elevated circulating levels of Type 1 (IFN , TNF- and IL-2), Type 2 (IL-5) and Type 17 (IL-17A) cytokines but decreased circulating levels of IL-22. This was associated with increased systemic levels of other pro-inflammatory cytokines (IL-1b, IL-6 and IL-18) and an anti- inflammatory cytokine (IL-10) but not Type 1 interferons. Moreover, TB antigen stimulated whole blood also showed increased levels of pro-inflammatory cytokines. Finally, Type1 and Type 17 cytokines in plasma exhibit a significant positive correlation with hemoglobin A1C (HbA1C) levels, indicating that impaired control of diabetes is associated with this pro inflammatory milieu. Multivariate analysis revealed that the association of pro-inflammatory cytokines with diabetes mellitus was not influenced by age, sex or other metabolic parameters (Fig. 6.5).

Conclusion: Therefore, our data reveal that TB with diabetes is characterized by heightened cytokine responsiveness, indicating that chronic inflammation underlying Type 2 diabetes potentially contributes to increased immune pathology and poor control in TB infection.



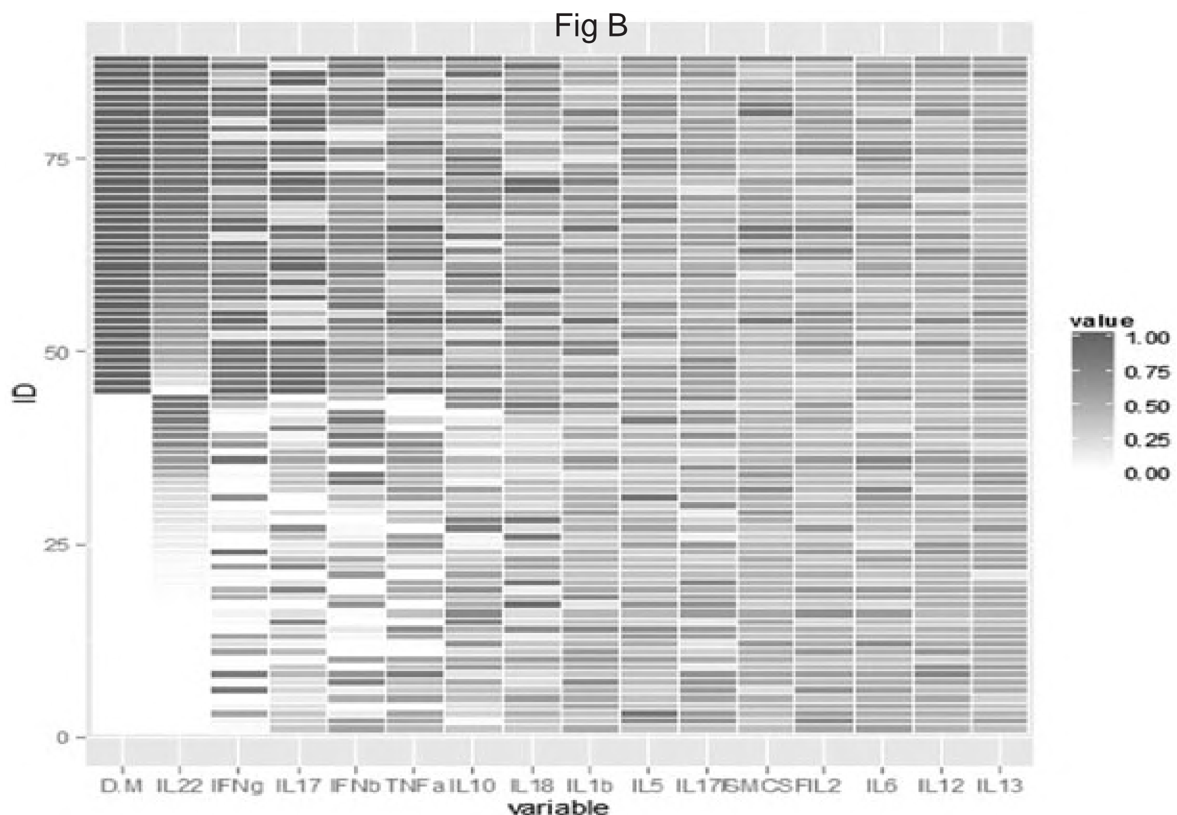


Fig. 6.5: Discriminatory value of cytokines in TB infected individuals

(A) The contribution of IFNg and IL-22 to the discrimination of diabetes from no diabetes in TB infected individuals (n=88) at a p value of < 0.05 is plotted using a logistic model. The red dots are those with diabetes and active PTB. The lines are contours from the model, representing the probability that an individual is diabetic.

(B) Heat map of predicted values from logistic regressions with a single cytokine predicting diabetes. The first column is the diabetes status (dark blue=DM, white=no DM). The rows are subjects sorted first by diabetes status, then by predicted diabetes status by IL-22, the best predictor. We have only shown cytokines with unadjusted p-values less than 0.05.

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Studies in progress:

(I) Characterization of immune responses in filarial-TB co-infection

We are studying the influence of filarial infetion on the immunological responses to TB antigens in latent TB infected individuals. This study is being conducted as a prospective case-control study in Kanchipuram district, Tamil Nadu. We are screening individuals by tuberculin skin test and Quantiferon-in-Tube Gold assay to detect latent TB and ELISA to detect filarial infections. We will perform whole blood cell cultures and multi-parameter flow cytometry to determine the immunological consequences of co-infection. We have performed *ex vivo* phenotyping on a variety of T, B, NK, DC and monocyte markers including regulatory T-cells, plasmacytoid and lymphoid dendritic cells, regulatory B cells and inflammatory monocytes on all our samples. We plan to recruit 60 patients and controls in this study and recruitment and follow up is ongoing.

(Contact person: Dr. S. Subash Babu, email: sbabu@nirt.res.in)

(ii) Characterization of immune responses in treatment induced latency in PTB

The immune responses in latent TB are poorly understood. While it is difficult to define the onset of latency during natural infection, patients undergoing treatment for TB are driven into a state of latency or cure. The present study on the effect of 3 and 4 month regimens containing MFX in sputum smear and culture positive PTB offers us the opportunity to study definitive immune responses pre and post treatment. We are evaluating a variety of innate and adaptive immune responses in patients before and after treatment and our study is comparing the differences in immunophenotype (eg. Markers of T, B and NK cell activation, proliferation and regulatory phenotype) and function (eg. production of cytokines, proliferative responses to TB antigens) at different time points following treatment. The kinetics of immune responses in patients who relapse is used to assess immunological predictors of relapse in TB. In addition, we are also trying to determine immunological differences between PTB, EPTB, LTB and uninfected individuals. We have performed *ex vivo* phenotyping on a variety of T, B, NK, DC and monocyte markers including regulatory T-cells, plasmacytoid and lymphoid dendritic cells, regulatory B cells and inflammatory monocytes on all our samples. We have recruited over 160 patients in this study thus far and recruitment and follow up is ongoing.

(Contact person: Dr. S. Subash Babu, email: sbabu@nirt.res.in)

AWARDS RECEIVED

Honour : Minister for Higher Education Mr. P. Palaniappan handing over the award to Dr. Soumya Swaminathan, at the Tamil Nadu Scientist Award function organised by the Tamil Nadu State Council for Science and Technology (TNSCST) in Chennai on Friday 19, October 2012. Also seen are Mr. Ramesh Chand Meena, Commissioner of Technical Education, and Dr. P. Kalairaj, Vice Chancellor, Anna University

Dr. P. Venkatesan, Scientist E, is being honoured with "**Lifetime Achievement Award**" by "The Tamil Nadu Dr. MGR Medical University", Chennai, in due recognition of his contribution to Medical Education and Health Research, during the Silver Jubilee Celeberations on November 24, 2012

UPGRADED FACILITIES

CULTURE ROOM

FLOW CYTOMETER CUM SORTER

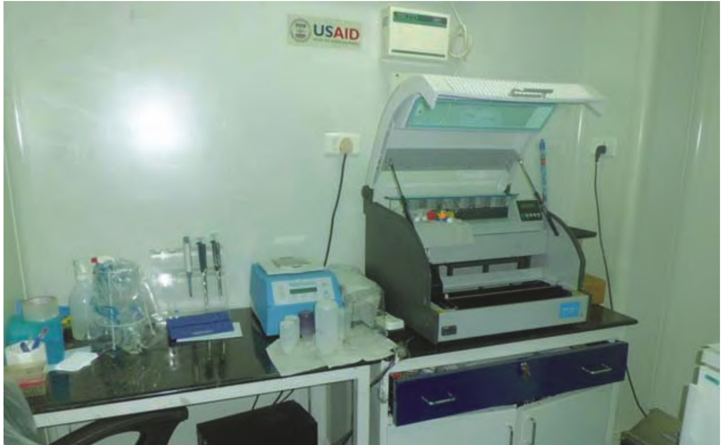
Auto Claves

COBAS Amplicor

BD BACTEC MGIT 960
Mycobacteria Culture System

Beckman Coulter AcT
5diff CP Hematology Analyzer

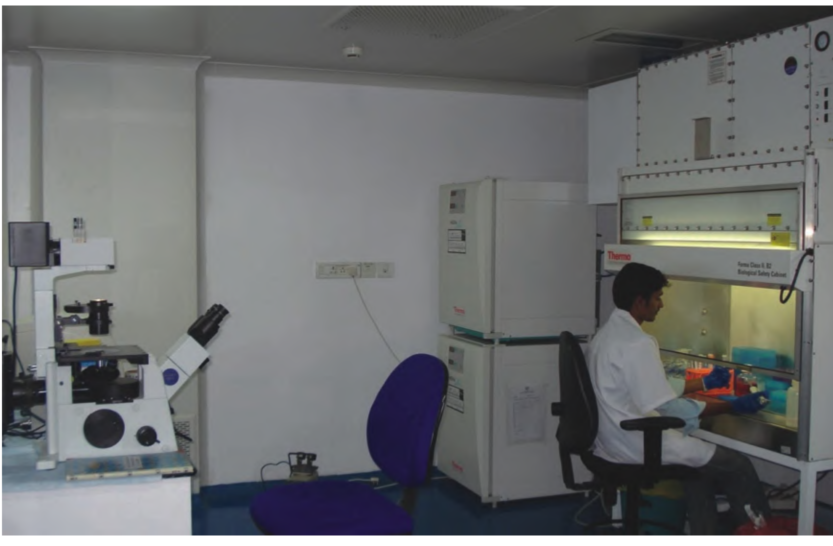
LINE PROBE ASSAY FACILITY



Applied Biosystems Gene Amp PCR System 9700

Directly Observed Treatment Short-course (DOTS)

BSL2+ CLASS 10000



Community based work

From left : Dr. Soumya Swaminathan, Director, NIRT, Dr. Lakshmi Murali, State TB officer, Dr. Kanagasabai, Dean, MMC, Dr. J. Radhakrishnan, Health Secretary, Tamilnadu State, Dr. Meenakshi, Director, Institute of Thoracic Medicine, Chennai.

World TB Day Symposium on Drug-Resistant Tuberculosis: Challenges and way forward – March 16, 2013

Health Secretary Dr. J. Radhakrishnan presenting a memento to a special invitee

FOGARTY REGIONAL HIV-TB MEETING

New TB and TB/HIV Multidisciplinary Research Partnership in India : Priorities and Opportunities

Venue : Chest Research Foundation, Pune, India

Oct 27-28, 2012

Inauguration by Director General, ICMR

From Left : Dr Soumya Swaminathan, Director, NIRT, Dr VM Katoch, Secretary DHR and DG, ICMR and Dr Christine Wanke, Professor of Medicine, Tufts University, USA

Participants

Group Photo

WORKSHOP ON "TRIPLE TROUBLE" : MALNUTRITION, TB & HIV
August 2 - 4, 2012 at Chennai

7

LIBRARY & INFORMATION SERVICES

The aim of the centre is to provide a modern and efficient Library service for all members of the NIRT and neighborhood institutes with valid reasons to make better use of the NIRT Library. Further it dedicated to meeting the research needs of the scientists of NIRT.

DIGITAL LIBRARY:

NIRT Library has its own homepage (http://digitallibrary.trcicer. res.in/digilib) provides web-based access to its resources, includes e-journals, e-books, and databases through intranet. It points to locate the back volume collections. It provides gateway to ICMR consortium journals, ICMR Resource sharing portal (JCCC@ICMR), open access resources, specialized databases, and international TB links. It enhances the customized integrated (24 hrs) access facility to the users.

STRATEGIC PLAN:

The NIRT has crossed thousands of publications as its assets. To keep all those archival collections and expanding access to technology for effective search and retrieval, it is planned to setting up an Institutional Repository at NIRT Library. A high end server has been procured for the purpose.

INFORMATION ALERT SERVICE:

The Library continues to alert the users about the latest information on TB with press clippings, latest online Journal (issue) published, newly added journals on Open Access Platform, forthcoming conference, seminars, symposium, workshops etc.

TRIAL ACCESS:

Trial access has been made for "Henry Steward Talk" on 'The Biomedical and Life Science Collection' for the Scientists of NIRT.

PUBLICATIONS:

A monthly publication "**TB Alert**" is being published among ICMR institutes. In order to expand the service, it has been started publishing to all the district TB officers.

(Contact person: Mr.R. Rathinasabapati, email: rrathinasabapati@nirt.res.in)

8
ELECTRONIC DATA PROCESSING

The mission of the Electronic Data Processing division is to provide the data entry/verification support for the research in tuberculosis studies conducted in clinic, epidemiology, laboratory, operational research and TB control program activities, and supports for data management, prepares pre-printed forms and reports for field activity of epidemiological surveys, and generates data tabulations, data analysis and helps in publication of research work. Accessing scientific journals and communications through e-mailing are the key requirements for our research organization. The existing IT equipments are being maintained under comprehensive annual maintenance contract. This includes managing the IT related facilities and ensuring that the IT equipments are maintained and kept up to-date. The management of functionality of LAN facility is carried out with the support given by NIH-ICER project. The video conferencing facilities are maintained by project staff attached to ICMR-HCL project and NIH-ICER project.

Highlights of the year

- Established National Knowledge Network facility with high bandwidth internet line (primary line) provided by Ministry of Communications, Government of India
- Replaced the old router by a new CISCO high-end router with built-in firewall
- Added 65 users with e-mail access facility during the year
- Wireless (Wi-Fi) configured for 11 users' laptops
- Replaced old 15 Laser printers by new printers
- Added two network laser printers and replaced two old network laser printers by new printers
- Data analysis completed and published a research report on 'a trend in TB infection prevalence'.

The quantum of documents of epidemiological, clinical, laboratory and program based studies entered and verified from April, 2012 to March, 2013 is shown below.

No. of documents entered: 60752; No. of documents verified: 64282.

A total of 48055 records were processed for the Chennai disease prevalence survey undertaken by epidemiology unit of NIRT. Data analysis was completed for the tuberculin skin test surveys conducted during 1999 to 2010 to study a trend in TB infection and a research report was published.

(Contact person: Mr.R. Subramani, email: subramanir@nirt.res.in)

APPENDICES

LIST OF PUBLICATIONS

Publications in Journals		**: 89**
Published	i) International	: 75
	ii) National	: 14
Books		: 3
Accepted	i) International	: 17
	ii) National	: 3

International:

1. Anuradha R, George JP, Pavankumar N, Kumaraswami V, Nutman TB, Babu S. Altered circulating levels of matrix metalloproteinases and inhibitors associated with elevated type 2 cytokines in lymphatic filarial disease. *PLoS Negl Trop Dis*. 2012;6:e 1681.
2. Anuradha R, George PJ, Pavan Kumar N, Fay MP, Kumaraswami V, Nutman TB, Babu S. Circulating microbial products and acute phase proteins as markers of pathogenesis in lymphatic filarial disease. *PLoS Pathog.*2012;8:e1002749.
3. Aravindhan V, Mohan V, Surendar J, Muralidhara Rao M, Anuradha R, Deepa M, Babu S.Effect of filarial infection on serum inflammatory and atherogenic biomarkers in coronary artery disease (CURES-121). *Am J Trop Med Hyg.*2012;86:828-833.
4. Azger D, Balaji S, Gomathi NS, Selvakumar N, Kumar V. Diagnostic luciferase reporter phage assay for activeand non-replicating persistors to detect tubercle bacilli from sputum samples. *Clin Microbiol Infect.*2012;18: 492-496.
5. Azger D, Nagarajan P, Das D, Kumar V, Selvakumar N. Retrieval of *Mycobacterium tuberculosis* cultures suspended in phosphate buffered saline. *Int J Mycobacteriol.* 2012;1:149-151.
6. Babu S, Anuradha R, Pavan Kumar N, George PJ, Kumaraswami V, Nutman TB. Toll- Like receptor- and filarial antigen-mediated, mitogen-activated protein kinase- and NF-^{0}B-dependent regulation of angiogenic growth factors in filarial lymphatic pathology. *Infect Immun.*2012;80:2509- 2518.
7. Babu S, Nutman TB. Immunopathogenesis of lymphatic filarial disease. *Semin Immunopathol .*2012;34:847-861.
8. Balaji S, Gomathi S, Azger D, Selvakumar N, Kumar V. Phage lysin as a substitute antibiotics to detect *Mycobacterium tuberculosis* from sputum samples with the BACTEC MGIT 960 system. *Clin Microbiol Infect.* 2012; 18:497-501.
9. Basirudeen SAK, Paulkumaran P, Raja A. Interferon gamma and interferon gamma inducible protein-10 in detecting tuberculosis infection. *J Infect.* 2012;64:73-579.
10. Cuevas LE, Browning R, Bossuyt P, Casenghi M, Cotton MF, Cruz AT, Dodd LE, Drobniewski F, Gale M, Graham SM,Grzemska M, Heinrich N, Hesseling AC, Huebner R, Jean-Philippe P, Kabra SK, Kampmann B, Lewinsohn D, Li M, Lienhardt C, Mandalakas AM, Marais BJ, Menzies HJ, Montepiedra G, Mwansambo C, Oberhelman R, Palumbo P, Russek-Cohen E, Shapiro DE, Smith B, Soto-Castellares G,

Starke JR, Swaminathan S, Wingfield C, Worrell C. Evaluation of tuberculosis diagnostics in children: 2. Methodological issues for conducting and reporting research evaluations of tuberculosis diagnostics for intrathoracic tuberculosis in children. Consensus from an expert panel. *J Infect Dis.*2012;205:S209-S215.

11. Cuevas LE, Petrucci R, Swaminathan S. Tuberculosis diagnostics for children in high burden countries: what is available and what is needed. Paediatr *Int Child Health.*2012;32:S30-S37.

12. Das D, Selvakumar N. Can LED fluorescence microscopy replace Ziehl-Neelsen microscopy in tuberculosis detection? *Int J Tuberc Lung Dis.* 2012;16:1558.

13. De Majumdar S, Vashist A, Dhingra S, Gupta R, Singh A, Challu VK, Ramanathan VD, Kumar P, Tyagi JS. Appropriate DevR (DosR)-mediated signaling determines transcriptional response, hypoxic viability and virulence of *Mycobacterium tuberculosis*. *PLoS One.*2012;7:e35847.

14. Ganga Devi NP, Ramesh K, Sudha S, Ponnuraja C, Padmapriyadarsini C, Swaminathan S. $CD4^+$ T-lymphocyte count/$CD8^+$ T-lymphocyte count ratio: surrogate for HIV infection in infants? J Trop Pediatr. 2012;58:394-397.15. George PJ, Anuradha R, Kumar NP, Kumaraswami V, Nutman TB, Babu S. Evidence of microbial translocation associated with perturbations in T-cell and antigen-presenting cell homeostasis in hookworm infections. *PLoS Negl Trop Dis.*2012;6:e1830.

16. Gomathi NS, Devi SM, Lakshmi R, Ranjani R, Wares DF, Kumar V, Selvakumar N. Capilia test for identification of *Mycobacterium tuberculosis* in MGIT™-positive cultures. *Int J Tuberc Lung Dis.* 2012;16: 788-792.

17. Graham SM, Ahmed T, Amanullah F, Browning R, Cardenas V, Casenghi M, Cuevas LE, Gale M, Gie RP, Grzemska M, Handelsman E, Hatherill M, Hesseling AC, Jean-Philippe P, Kampmann B, Kabra SK, Lienhardt C, Lighter-Fisher J, Madhi S, Makhene M, Marais BJ, McNeeley DF, Menzies H, Mitchell C, Modi S, Mofenson L, Musoke P, Nachman S, Powell C, Rigaud M, Rouzier V, Starke JR, Swaminathan S, Wingfield C. Evaluation of tuberculosis diagnostics in children: 1. Proposed clinical case definitions for classification of intrathoracic tuberculosis disease. Consensus from an expert panel.*J Infect Dis.*2012;205:S199-S208.

18. Hassan S, Debnath A, Vasantha M, Hanna LE. Computational structural analysis of proteins of *Mycobacterium tuberculosis* and a resource for identifying off-targets. *J Mol Model.*2012;18:3993-4004 and Erratum p.no.4005.

19. Hemanth Kumar AK, Sudha V, Ramachandran G. Simple and rapid liquid chromatography method for simultaneous determination of isoniazid and pyrazinamide in plasma. *SAARC J Tuberc Lung Dis HIV/AIDS.*2012;9:26-29.

20. Hilda, JN, Selvaraj A, Das SD *Mycobacterium tuberculosis* H37Rv is more effective compared to vaccine strains in modulating neutrophil functions: An *in vitro* study. *FEMS Immunol Med Microbiol.*2012;66:372–381

21. Joseph J, Hassan S, Rajendran V, Kumar V. Functional assignment of the 64 mycobacteriophages into gene families. *Asian Pacific J Trop Biomed.* 2012; (Supplement 1) : S 383-385.

22. Kannan P, Malini V, Narayanan S. Characterization of Ffh of *Mycobacterium tuberculosis* and its interaction with 4.5S RNA. *Microbiol Res.* 2012;167: 520-525.

23. Kolappan C, Subramani R, Chandrasekaran V, Thomas A. Trend in tuberculosis infection prevalence in a rural area in south India after implementation of the DOTS strategy. *Int J Tuberc Lung Dis.*2012;16:1315-1319.

24. Kumar A, Kumar AMV, Gupta D, Kanchar A, Mohammed S, Srinath S, Tripathy S, Rajasekaran S, Chan PL, Swaminathan S, Dewan PK. Global guidelines for treatment of tuberculosis among persons living with HIV: unresolved issues. *Int J Tuberc Lung Dis.*2012;16:573-578.

25. Kumar SR, Gopalan N, Patrawalla P, Menon P, Mayer K, Swaminathan S. Immune reconstitution inflammatory syndrome in HIV-infected patients with and without prior tuberculosis. *Int J STD AIDS.*2012;23:419-423.

26. Kumar D, Narayanan S. pknE, a serine / threonine kinase of Mycobacterium tuberculosis modulates multiple apoptotic paradigms. *Infect Genet Evol.* 2012;12:737-747.

27. Kumar SR, Gopalan N, Patrawalla P, Menon P, Mayer K, Swaminathan S. Immune reconstitution inflammatory syndrome in HIV-infected patients with and without prior tuberculosis. *Int J STD AIDS.*2012;23:419-423.

28. Lagrange PH, Thangaraj SK, Dayal R, Despande A, Ganguly NK, Girardi E, Joshi B, Katoch K, Katoch VM, Kumar M, Lakshmi V, Leportier M, Longuet C, Malladi SV, Mukerjee D, Nair D, Raja A, Raman B, Rodrigues C, Sharma P, Singh A, Singh S, Sodha A, Basirudeen SAK, Vernet G, Goletti D. A toolbox for tuberculosis diagnosis: An Indian multicentric study (2006-2008): Microbiological results. *PLoS One.*2012;7:e43739.

29. McNerney R, Maeurer M, Abubakar I, Marais B, McHugh TD, Ford N, Weyer K, Lawn S, Grobusch MP, Memish Z, Squire SB, Pantaleo G, Chakaya J, Casenghi M, Migliori GB, Mwaba P, Zijenah L, Hoelscher M, Cox H, Swaminathan S, Kim PS, Schito M, Harari A, Bates M, Schwank S, O'Grady J, Pletschette M, Ditui L, Atun R, Zumla A. Tuberculosis diagnostics and biomarkers: needs, challenges, recent advances, and opportunities. *J Infect Dis.*2012;205:S147-S158.

30. Mohan SB, Ravi Kumar BV, Dinda SC, Naik D, Prabu Seenivasan S, Kumar V, Rana DN, Brahmkshatriya PS. Microwave-assisted synthesis, molecular docking and antitubercular activity of 1,2,3,4-tetrahydropyrimidine-5-carbonitril derivatives *Bioorg Med Chem Lett.*2012;22:7539-7542.

31. Pho MT, Swaminathan S, Kumarasamy N, Losina E, Ponnuraja C, Uhler LM, Scott CA, Mayer KH, Freedberg KA, Walensky RP. The cost-effectiveness of tuberculosis preventive therapy for HIV-infected individuals in southern India: A trial-based analysis. *PLoS One.*2012;7:e36001.

32. Radhakrishnan M, Priya J, Balagurunathan R, Kumar V. Miniaturized fermentation in eppendorf tubes for the detection of antagonistic actinomycetes. *Int J Bioassays.* 2012;1:30-35.

33. Rajendran V, Hassan S, Joseph J, Selvakumar N, Kumar V. *In silico* sequence and structure analysis for mycobacteriophages. *Asian Pacific J Trop Biomed.* 2012; (Supplement 1):S377-S379.

34. Ramana Rao PV, Sikhamani R, Raja A. Effect of recombinant cytokines on the expression of natural killer cell receptors from patients with TB or/ and HIV infection. *PLoS One.*2012;7:e37448.

35. Ramachandran G, Swaminathan S. Role of pharmacogenomics in the treatment of tuberculosis: a review. *Pharmacogenomics Pers Med.*2012; 5:89–98.

36. Ramakrishnan K, Shenbagarathai R, Kavitha K, Thirumalaikolundu-subramanian P, Rathinasabapati R.Selenium levels in persons with HIV/ tuberculosis in India, Madurai city. *Clin Lab.*2012;58:165-168.

37. Ramamurthy D, Venkatesan P. Stochastic model of the growth of human immuno deficiency virus in infected individuals. *Int J Comput Mathemat App.* 2012;6:79-88.

38. Rao VG, Gopi PG, Bhat J, Yadav R, Selvakumar N, Wares DF. Selected risk factors associated with pulmonary tuberculosis among Saharia tribe of Madhya Pradesh, central India. *Eur J Public Health.*2012;22:271-273.

39. Rao VG, Bhat J, Yadav R, Gopi PG, Selvakumar N, Bhondeley MK, Anjinappa SM, Ramachandra J, Chadha VK, Wares F. Prevalence of pulmonary tuberculosis – A baseline survey in central India. PLoS One. 2012;7:e43225.40.Refaya AK, Sivakumar S, Sundararaman B, Narayanan S. Polymorphism in the RD1 locus and its effect on downstream genes among south Indian clinical isolates of *Mycobacterium tuberculosis*. *J Med Microbiol.*2012;61:1352-1359.

41. Schmidt C, Smith C, Barin B, Bakhtyari A, Bart PA, Bekker LG, Chomba E, Clumeck N, Ho D, Hoosen A, Jaoko W, Kaleebu P, Karita E, Keefer MC, van Lunzen J, McMichael A, Mehendale S, Peters B, Ramanathan VD, Robinson A, Rockstroh J, Vardas E, Vets E, Weber J, Graham BS, Than S, Excler JL, Kochhar S, Ho M, Heald A, Fast PE. Background morbidity in HIV vaccine trial participants from various geographic regions as assessed by unsolicited adverse events. *Hum Vaccin Immunother.*2012;8:630-638.

42. Selvaraj P, Harishankar M, Singh B, Banurekha VV, Jawahar MS. Effect of vitamin D_3 on chemokine expression in pulmonary tuberculosis. *Cytokine.*2012;60:212-219.

43. Selvakumar N, Syam Sunder A, Gomathi S, Ponnuraja C, Kumar V. Performance indicators of fluorescence microscopy for sputum samples in pulmonary tuberculosis. *Int J Mycobacteriol.*2012;1:143-145.

44. Selvakumar N, Silambuchelvi K, Gomathi S, Syam Sunder A, Anbarasu S, Banu Rekha V, Ponnuraja C, Kumar V. Qualityindicators in a mycobacteriology laboratory supporting clinical trials for pulmonary tuberculosis. *Int J Mycobacteriol.*2012;1:185-189.

45. Senbagavalli P, Hilda JN, Ramanathan VD, Kumaraswami V, Nutman TB, Babu S. Immune complexes isolated from patients with pulmonary tuberculosis modulate the activation and function of normal granulocytes. *Clin Vaccine Immunol.*2012;19:1965-1971.

46. Sreenivas K, Vijayan K, Babu S, Narayanan RB. Recombinant *Brugia malayi* pepsin inhibitor (rBm33) induced monocyte function and absence of apoptotic cell death: An *in vitro* study. *Microb Pathog.*2012;53:19-27.

47. Sundaramurthi JC, Brindha S, Shobitha SR, Swathi A, Ramanandan P, Hanna LE. In silico identification of potential antigenic proteins and promiscuous CTL epitopes in Mycobacterium tuberculosis. *Infect Genet Evol.*2012;12:1312-1318.

48. Sundaramurthi JC, Swaminathan S, Hanna LE. Resistance-associated epitopes of HIV-1C-highly probable candidates for a multi-epitope vaccine. *Immunogenetics.* 2012;64:767-772.

49. Sundaram N, Venkatesan P. Bayesian regression approach for modeling response time in clinical trials. *Int J Comput Mathemat App.*2012;6:65-78.

50. Surendar J, Mohan V, Pavankumar N, Babu S, Aravindhan V. Increased levels of serum granulocyte-macrophagecolony-stimulating factor is asssociated with activated peripheral dendritic cells in type 2 diabetes subjects (CURES-99). *Diabetes Technol Ther*.2012; 14:344-349.

51. Swaminathan S, Menon PA, Narendran G, Venkatesan P, Ramesh Kumar S, Ranjani R, Ponnuarja C, Sheik I, Padmapriyadarsini C, Ganga Devi NP, Elangovan T, Pho MT, Wares F, Narayanan PR. Efficacy of a six-month versus a 36-month regimen for prevention of tuberculosis in HIV-infected persons in India: A Randomized clinical trial. *PLoS One.* 2012 ;7:e47400.

52. Swaminathan S, Rekha VB. Antigen detection as a point-of-care test for TB: the case of lipoarabinomannan. *Future Microbiol.*2012;7:559-564.

53. Swetha S, Babu S, Das SD. Comparison of localized versus systemic levels of Matrix metalloproteinases (MMPs), its tissue inhibitors (TIMPs) and cytokines in tuberculous and non-tuberculous pleuritis patients. *Hum Immunol.*2012;73:985-991.

54. Thomas B, Mimiaga MJ, Mayer KH, Perry NS, Swaminathan S, Safren SA. The influence of stigma on HIV risk behavior among men who have sex with men in Chennai, India. *AIDS Care.* 2012;24:1401-1406.

55. Thomas B, Mimiaga MJ, Mayer KH, Johnson CV, Closson EF, Menon S, Mani J, Vijaylakshmi R, Dilip M, Betancourt T, Safren SA. Ensuring it works: A community-based approach to HIV prevention intervention development for men who have sex with men in Chennai, India. *AIDS Edu Prev.*2012;24:483-499.

56. Venkatesan P, Premalatha V. Genetic-neuro approach for disease classification. *Int J Sci Technol.*2012;2:473-478.

57. Venkatesan P, Meena Devi G. Disease classification by least squares support vector machine. *Int J Comput Mathemat App.*2012;6:105-112.

58. Zumla A, Abubakar I, Raviglione M, Hoelscher M, Ditiu L, McHugh TD, Squire SB, Cox H, Ford N, McNerney R, Marais B, Grobusch M, Lawn SD, Migliori GB, Mwaba P, O'Grady J, Pletschette M, Ramsay A, Chakaya J, Schito M, Swaminathan S, Memish Z, Maeurer M, Atun R. Drug-resistant tuberculosis—current dilemmas, unanswered questions, challenges, and priority needs. *J Infect Dis.*2012;205:S228-S240.

2013:

59. Anbarasu D, Ponnuraja C, Raja A. Multiplex analysis of cytokines/chemokines as biomarkers that differentiate healthy contacts from tuberculosis patients in high endemic settings. *Cytokine.*2013;61:747-754.

60 Anbarasu D, Prabhavathi M, Raja A. Comparison of whole blood and PBMC assays for T-cell functional analysis. *BMC Res Notes.*2013;120.

61. Balaji S, Gomathi S, Azger D, Kumar V. Phage lysin to control the overgrowth of normal flora in processed sputum samples for the rapid and sensitive detection of *Mycobacterium tuberculosis* by luciferase reporter phage assay. *BMC Infect Dis* 2013;13:44.

62. Chadha VK, Sarin R, Narang P, John KR, Chopra KK, Jitendra R, Mendiratta DK, Vohra V, Shashidhara AN, Muniraj G, Gopi PG, Kumar P. Trends in the annual risk of tuberculous infection in India. *Int J Tuberc Lung Dis.*2013;17:312-319.

63. Graham SM, Casenghi M, Jean-Philippe P, Hatherill M, Hesseling AC, Nachman S, Starke JR, Swaminathan S, Cuevas LE. Reply to Holm *et al. J Infect Dis.*2013;207:871-872.

64. Kumar D, Kannan P, Challu VK, Kumar P, Narayanan S. PknE, a serine/threonine protein kinase from *Mycobacterium tuberculosis* has a role in adaptive responses. *Arch Microbiol.*2013;195:75-80.

65. Lakshmi R, Ranjani R, Syam Sundar A, Rehman F, Radhika G, Kumar V. Optimization of the conventional minimum inhibitory concentration method for drug susceptibility testing of ethionamide. *Int J Mycobacteriol.*2013;2:29-33.

66. Mehendale S, Thakar M, Sahay S, Kumar M, Shete A, Sathyamurthi P, Verma A, Kurle S, Shrotri A, Gilmour J, Goyal R, Dally L, Sayeed E, Zachariah D, Ackland J, Kochhar S, Cox JH, Excler JL, Kumaraswami V, Paranjape R, Ramanathan VD. Safety and immuno genicity of DNA and MVA HIV-1 subtype C vaccine prime-boost regimens: A phase I randomised trial in HIV-uninfected Indian volunteers. *PLoS One.*2013;8:e55831.

67. Pavan Kumar N, Sridhar R, Banurekha VV, Nair D, Jawahar MS, Nutman TB, Babu S. Expansion of pathogen-specific mono and multifunctional Th1 and th17 cells in multi-focal tuberculous lymphadenitis. *PLoS One.*2013; 8:e57123.

68. Pavan Kumar N, Gopinath V, Sridhar R, Hanna LE, Banurekha VV, Jawahar MS, Nutman TB, Babu S. IL-10 dependent suppression of type 1, type 2 and type 17 cytokines in active pulmonary tuberculosis. *PLoS One.*2013;8: e59572.

69. Radhakrishnan M, Gopikrishnan V, Suresh A, Selvakumar N, Balagurunathan R, Kumar V. Characterization and phylogenetic analysis of antituberculous compound producing actinomycete strain D25 isolated from Thar Desert soil, Rajasthan. *Bioinformation.*2013;9:18-22

70. Radha RK, Vekatesan P. Bayes estimator as a function of some classical estimator for power function distribution. *Int J Stats Analysis.*2013;3:105-109.

71. Ragunath PK, Ravi Mohan R, Venkatesan P. A study on relevance of student's attitude, implementing an interdisciplinary approach in a post graduate program. *Procedia Computer Science*;2013;18:218-229.

72. Rekha B, Jagarajamma K, Chandrasekaran V, Wares F, Sivanandham R, Swaminathan S. Improving screening and chemoprophylaxis among child contacts in India's RNTCP: A pilot study. *Int J Tuberc Lung Dis.*2013;17:163-168.

73. Suhadev M, Mahadevan U, Thomas BE, Swaminathan S. Perceived social support among the spouses of men living with HIV infection. *Int J Public Health Epid.*2013;2:66-71.

74. Sundaramurthi JC, Ramanathan VD, Hanna LE. HLA-B*27:05-Specific Cytotoxic T lymphocyte epitopes in Indian HIV type 1C. *AIDS Res Hum Retroviruses.*2013;29:47-53.

75. Venkatesan P, Thomas T. A comparative study of neural network architectures for predicting gene expression in *M. tuberculosis*. *Int J Pharma Biosci.*2013;4:965-972.

National:

1. Gomathi S, Rehman F, Kumar V, Selvakumar N. Equivalence of acid alone or acid-alcohol as decolourizing agent in Ziehl-Neelsen method. *Indian J Tuberc.*2012;59:219-223.

2. Kumar D, Narayanan S. PknE, a Serine / Threonine kinase from *Mycobacterium tuberculosis* modulates apoptosis and arginase signaling in macrophages. *Indian J Genet Mole Res.*2012;1:20.

3. Lakshmi R., Kumar V, Rahman F, Ranjani R. Consistency of standard laboratory strain *Mycobacterium tuberculosis* H37Rv with ethionamide susceptibility testing. *Indian J Med Res.*2012;135:672-674.

4. Manivannan S, Rao NV, Ramanathan VD. Role of complement activation and antibody in the interaction between *Mycobacterium tuberculosis* and human macrophages. *Indian J Exp Biol.*2012;50:542-550.

5. Muthuswamy M, Prabuseenivasan, Kumar V. Antibacterial activity of seleced medicinal plants used by the tribal people. *Biomedicine.*2012; 32:199-206.

6. Padmapriyadarsini C, Thomas A. Tuberculosis infection control practices in healthcare settings. *J Indian Med Assoc.*2012;110:112-114.

7. Raghu B, Venkatesan P. Relationship between cigarette smoking and novel risk factors for cardiovascular disease. *J Biomed Sci.*2012;1.

8. Ramachandran G, Hemanth Kumar AK, Srinivasan R, Geetharani A, Sugirda P, Nandhakumar B, Nandini R, Tharani CB. Effect of rifampicin & isoniazid on the steady state pharmacokinetics of moxifloxacin. *Indian J Med Res.*2012;136:979-984.

9. Rekha VV, Rajaram K, Kripasankar AS, Parthasarathy R, Umapathy KC, Sheikh I, Selvakumar N, Victor M, Niruparani C, Sridhar R, Jawahar MS. Efficacy of the 6-month thrice-weekly regimen in the treatment of new sputum smear-positive pulmonary tuberculosis under clinical trial conditions. *Natl Med J India.* 2012;25:196-200.

10. Shyamala Gopu G, Rao VB, Vadivelu J. Impact of health education on the knowledge of TB among sputumpositive pulmonary tuberculosis patients and their care givers. *The Nursing Journal of India.*2012;CIII-160-162.

11. Tintu T, Venkatesan P. Classification of microarray data using support vector machine. *E J Pharmacol Therapy.*2012;5:25-28.

12. Venkatesan P, Ramamurthy D, Sundaram N. HIV/AIDS projection in Tamilnadu using back calculation method. *Indian J Sci Technol.*2012; 5:3157-3162.

2013:

13. Venkatesan P, Yamuna NR. Treatment response classification in randomized clinical trials: A decision tree approach. *Indian J Sci Technol.* 2013;6:74-79.

14. Venkatesan P, Mullai M. Clustering of disease data base using self organized maps and logical inference. *Indian J Automation Artificial Intelligence.*2013;1:2-6.

Chapters

1. Ganga Devi NP, Rekha VV, Swaminathan S. Tuberculosis and HIV co-infection in children. Book entitled, “Principles of Perinatal and Pediatric HIV/AIDS” - First edition, publisher Jaypee, 2012: 239-252.

2. Jawahar MS, Rekha VV. Drugs used in tuberculosis and leprosy. In J.K.Aronson, editor: Side Effects of drugs Annual, Amsterdam: Netherlands, 2012;34:479-486.

3. Selvaraj K, Alagarasu K, Raghavan S. Genetics of susceptibility to mycobacterial disease. Published On line January 2013. In: eLS. John Wiley & Sons, Ltd: Chichester. (DOI:10.1002/9780470015902.a0023875).

Accepted:

International:

1. Anbarasu D, Raja CP, Raja A. Multiplex analysis of cytokines/chemokines as biomarkers that differentiate healthy contacts from tuberculosis patients in high endemic settings. *Cytokine*.
2. Andrade BB, Pavan Kumar N, Mayer-Barber KD, Barber DL, Sridhar R, Rekha VV, Jawahar MS, Nutman TB, Sher A, Babu S. Plasma heme oxygenase-1 levels distinguish latent or successfully treated human tuberculosis from active disease. *PLoS One.*
3. Chandrasekaran P, Tripathy S, Sekar L, Bhavani PK, Gaikwad N, Annadurai S, Narendran G, Selvakumar N, Risbud AR, Sheta D, Rajasekaran S, Thomas A, Wares F, Swaminathan S. Evaluation of a diagnostic algorithm for sputum smear–negative pulmonary tuberculosis in HIV-infected adults. *J Acquir Immune Defic Syndr.*
4. Dayal PL, Alexander LT, Ponnuraja C, Rama R, Venkatesan P. Modelling of time to event breast cancer data using accelerated failure time in south India women. *Global Res Analysis Int.*2013;2(5):192-194.
5. George PJ, Anuradha R, Kumaran PP, Chandrasekaran V, Nutman TB, Babu S. Modulation of mycobacterial-specific Th1 and Th17 cells in latent tuberculosis by coincident hookworm infection. *J Immunol.*
6. Hemanth Kumar AK, Sudha V, Ramachandran G. Simple and rapid liquid chromatography method for determination of rifabutin in plasma. *SAARC J TB, Lung diseases & HIV/AIDS.*
7. Jawahar MS, Banurekha VV, Paramasivan CN, Rahman F, Ramachandran R, Venkatesan P, Balasubramanian R, Selvakumar N, Ponnuraja C, Iliayas AS, Gangadevi NP, Raman B, Baskaran D, Kumar SR, Kumar MM, Mohan V, Ganapathy S, Kumar V, Shanmugam G, Charles N, Sakthivel MR, Jagannath K, Chandrasekar C, Parthasarathy RT, Narayanan PR. Randomized clinical trial of thrice-weekly 4-month moxifloxacin or gatifloxacin containing regimens in the treatment of new sputum positive pulmonary tuberculosis patients. *PLoS One.*
8. Lakshmi R, Ranjani R, Devika K, Radhika G, Syam Sundar A, Rahman F, Selvakumar N, Kumar V. Modification of proportion sensitivity testing method for ethionamide. *World J Microbiol Biotechnol.*
9. Narendran G, Andrade BB, Porter BO, Chandrasekhar C, Venkatesan P, Menon PA, Subramanian S, Anbalagan S, Bhavani KP, Sekar S, Padmapriyadarshini C, Kumar S, Ravichandran N, Raja K, Bhanu K, Mahilmaran A, Sekar L, Sher A, Sereti I, Swaminathan S. Paradoxical tuberculosis immune reconstitution inflammatory syndrome (TB-IRIS) in HIV patients with culture confirmed pulmonary tuberculosis in India and the potential role of IL-6 in prediction. *PLoS One.*
10. Kumar NP, Sridhar R, Banurekha VV, Jawahar MS, Nutman TB, Babu S. Expansion of pathogen - specific Th1and Th17 cells in pulmonary tuberculosis with coincident type 2 diabetes mellitus. *J Infect Dis.*
11. Kumar NP, Anuradha R, Andrade BB, Suresh N, Ganesh R, Shankar J, Kumaraswami V, Nutman TB, Babu S. Circulating biomarkers of pulmonary and extra-pulmonary tuberculosis in children. *Clin Vaccine Immunol.*

12. Padmapriyadarsini C, Bhavani PK, Tang A, Hemanth Kumar AK, Ponnuraja C, Narendran G, Hannah LE, C. Ramesh C, Chandrasekhar C, Wanke C, Swaminathan S. Early changes in Hepatic functions among HIV/TB patients treated with Nevirapine or Efavirenz along with Rifampicin-based ATT. *Int J Infect Dis.*

13. Radha RK, Venkatesan P. On the double prior selection for the parameter of Maxwell distribution. *Int J Scientific & Eng Res.*

14. Radhakrishnan R, Prabuseenivasan S, Balaji S, Sankar U, Thomas A, Kumar V, Selvakumar N. Blinded re-checking of acid fast bacilli smears by light emitting diode microscopy. *Int J Tuberc Lung Dis.*

15. Ramachandran G, Hemanth Kumar AK, Bhavani PK, Poorana Gangadevi N, Sekar L, Vijayasekaran D, Banu Rekha VV, Ramesh Kumar S, Ravichandran N, Mathevan G, Swaminathan S. Age, nutritional status and INH acetylator status affect pharma cokinetics of anti-tuberculosis drugs in children. *I Int J Tuberc Lung Dis.*

16. Suba S, Narayanan S. The lpqS knockout mutant of Mycobacterium tuberculosis is attenuated in Macrophages. *Microbiol Res.*

17. Narayanan S, Deshpande U. Whole-Genome sequences of four clinical isolates of *Mycobacterium tuberculosis* from Tamil Nadu, South India. *Genome Announc.*

National:

18. Ramachandran G, Hemanth Kumar AK, Ponnuraja C, Ramesh K, Lakshmi R, Chandrasekharan C, Swaminathan S. Lack of association between plasma NNRTIs and virological outcomes during rifampicin co-administration in HIV-infected TB patients. *Indian J Med Res.*

19. Srinivasan R, Venkatesan P. Bayesian Kriging of Tuberculosis in Chennai: A Small Scale Analysis. *Indian J App Res.*

20. Harini L, Narayanan S. Comparative genomic analysis of mycobacteria (actinobacteria): redundancy in function among serine threonine protein kinases. *Global J Bio-Sci Biotechnol.*

AWARDS / HONOURS

Dr. Soumya Swaminathan

- Fellow of the National Academy of Sciences, Allahabad
- Fellow of the Indian Academy of Sciences, Bangalore
- Tamil Nadu award for Science and Technology – 2012

Dr. P. Venkatesan

Awarded with **"Lifetime Achievement Award"** by "The Tamilnadu Dr. MGR Medical University" Chennai on 24th November, 2012 (Silver Jubilee Year-2012) in recognition of his exemplary contribution to the cause of Medical Education & Health Services.

Mrs. Jayalakshmi Vadivel

Received the `Best Nurse Award' from the Tamil Nadu Dr. MGR Medical University, Chennai-32, during September 2012.

PATENT FILED

Dr. Sujatha Narayanan

A patent was filed on "Biosynthesis of a compound and its antimycobacterial activity".

CAPACITY BUILDING

I. Dr. Luke Elizabeth Hanna

Proficiency Testing:

- For DNA-PCR: Participated successfully in 4 rounds of panel testing with the NIH-VQA program and CDC-GAP.
- For Viral Load Assay: Participated successfully in 3 rounds of panel testing with the NIH-VQA program and RCPA-QAP.
- For HIV-1 Drug Resistance Genotyping: Participated successfully in 2 rounds of panel testing with the NIH-VQA program and WHO Accreditation Program.
- For CD4 count: Participated successfully in 4 rounds of testing with the NARI-QASI program.

Participation in External Quality Assurance Programs and Ongoing External:

I. "Qualitative Research Methods and Analysis and Instrument Development Workshop" at Bagayam, Vellore, 7th to 12th May 2012–Mr. R. Senthilkumar and Mrs. V. Sujatha.

II. "5 days Intensive Workshop on Tobacco Cessation Intervention" at Cancer Institute (WIA), Adyar, 13th - 18th August, 2012 – Mr. E. Thiruvalluvan, Mr. S. Senthil.

III. "Capacity building workshop on HIV/AIDS in the area of basic services" at Lalaram sarup institute of TB & Respiratory disease, Delhi - 24th - 28th September, 2012- Mrs. Chandra Suresh.

IV. "Qualitative Research Methods and Analysis and Instrument Development Workshop" at Bagayam, Vellore, during November 2012– Ms.A. Srividya, Ms. Basilea Watson and Ms. B. Priscilla Rebecca.

V. Capacity building workshop on "Operational research in Basic Services" held at LRS Insitutte of Chest Diseases, New Delhi during September 2012 – Ms.A. Srividya and Ms. Chandra.

SPECIAL ASSIGNMENTS

MEMBERSHIP IN COMMITTEES

Dr. Soumya Swaminathan

- Chair of the HIV section of the International Union Against TB and Lung Diseases
- Member of the TB steering committee of the IMPAACT network (NIH)
- Member, Childhood TB subgroup, DOTS Expansion Working Group, Stop TB Partnership
- Member, International Conference Organizing Committee, International AIDS Society Conference, Kuala Lumpur Founder member, Sentinel Project on Pediatric Drug Resistance TB
- Member, Country Coordination Mechanism (CCM) for the Global Fund – to fight against AIDS, Tuberculosis and Malaria Chair, Indo-Brazil working group on Biotechnology
- Member, Indo-Russian working group on Medical Research, DST
- Member, Expert Group on Pediatric TB, Central TB Division
- Member, National Technical Working Group (NTWG) on HIV/TB
- Member, Board of Studies on Research, Tamil Nadu Dr. MGR Medical University

Dr. Alamelu Raja

- Expert Member of the Institutional Review Board of Sri Kanchi Kamakoti CHILDS Trust Hospital
- Member, Editorial Board, Indian Journal of Medical Research.
- Life Member, Indian Immunology Society

Dr. P. Selvaraj

- Associate editor for International Journal of Tuberculosis and Lung Diseases (IJTLD) by invitation.

Dr. P. Venkatesan

- Adjunct Professor - Manipal University, Manipal.
- Honorary Visiting Professor - Sri Ramachandra Medical University, Chennai.
- Chairman - Institute Ethics Committee/Institute Review Board, Sri Ramachandra Medical University, Chennai.
- Chairman - Board of Studies: M.Sc., (Bioinformatics) and B.Tech., (Biomedical informatics)- Sri Ramachandra Medical University, Chennai.
- Expert Member - Scientific Advisory Board, Sai's Biosciences Research Institute, Chennai
- Member- Board of Studies: M.Sc., (Statistics) and M.Sc., (Biostatistics), University of Madras, Chennai.
- Member- Board of Studies – M.Phil., (Statistics), B.Sc., (Biostatistics) and MPH, Manonmaniam Sundaranar University, Tirunelveli.
- Member – Board of Studies: M. Phil., (Statistics) and M.Sc., (Statistics), Bharathidasan University, Tiruchirappall.
- Member- Board of Studies: M.Sc., (Mathematics), M.Phil (Mathematics) and M.Tech (Computer Science) Periyar University, Salem.

- Member- Board of Studies: M.Tech., (Bioinformatics) and B.Tech. (Bioinformatics), Sathyabama University, Chennai.
- Member- Board of Studies: M.Sc., (Biotechnology & Bioinformatics), SRM University, Chennai.
- Member- Board of Studies: M.Sc., (Mathematics), Meenakshi College for Women (Autonomous), Chennai.
- Member- Board of Studies: M.Sc., (Bioinformatics), Stella Maris College for Women (Autonomous), Chennai.
- Member – Institute Ethics Committee: ESI PG Institute of Medical Education & Research, Chennai.
- Member - Editorial Board: Journal of Pure and Applied Spectrophysics.
- Member - Editorial Board: Indian Journal of Science and Technology.
- General Secretary - Indian Society for Medical Statistics (ISMS).
- President - International Biometric Society (IR).

Dr. Paul Kumaran

- Member of Central internal evaluation for RNTCP for the state of Uttar Pradesh during April 2012.

Dr. Luke Elizabeth Hanna

- Member of Technical Advisory Group for Tamil Nadu State AIDS Control Society.

Dr. Beena E Thomas

- Member of the Board of studies – University of Madras – Social work
- Member of Institutional Review Board – GHTM (Govt. Hospital of Thoracic Medicine)
- Member of IRB India CLEN

CONFERENCES / SEMINARS / WORKSHOPS ORGANIZED

The centre has provided opportunities to its staff members (research and technical) and students for their professional development through participation in workshops, conferences, seminars and training programmes at national and international level. The summary of national and international level participation is given below:

At National level:

Conferences – 47

Workshops – 12

At International level:

Conferences – 15

Conferences/Workshops/Training Programmes Organized:

1. Workshop on "Tripe Trouble: Malnutrition, TB and HIV" (WS 89-2011, Triple Trouble) supported by Indo-US Science & Technology Forum (IUSSTF), New Delhi - at NIRT, during August 2-4, 2012.
2. Session on "An Update on TB diagnosis and management" and a panel discussion on enhancing contribution of Private Practitioners in TB control for the private practitioners in commemoration with the World TB day 2012 – at Chennai – during April 2012.
3. Workshop on "Ethical principles, Good Clinical Practices in clinical research and Update on the Drugs and Cosmetic rules" for the Ethics Committee members of NIRT and YRG Care at NIRT - during March, 2013.
4. Symposium on drug resistant TB : Challenges and way forward for World TB day jointly with Institute of Thoracic Medicine & Madras Medical College.
5. Dissemination Workshop on "Addressing psychosocial needs and HIV risk in Indian MSM", at NIRT - during April, 2012.
7. NSS Programme for School teachers and Lecturer's, professors, NSS coordinators for Tamilnadu and Puducherry – 21 No. of programmes during April 2012 - March 2013.
8. International Biometric Society Indian Region – 2012 of National workshop on "Statistical methods for clinical trials" during December 2012.
9. Research Methodology workshop for Human Reproduction Research centres (HRRCs) Field units of ICMR at NIRT, during December 2012.

Ph.D. Scholars

List of staff / students who have obtained their Ph.D. degree (Part time/Full time) from the University of Madras

Sl.No.	Name of the Candidate	Title of the Ph.D. thesis	Part / Full time	Supervisor /Guide
1.	Mr.R. Rathinasabapati	Institutional repository of the NIRT	Part time	Dr.A. Amudhavalli

List of staff/students who have submitted their Synopsis / Thesis and waiting for their Ph.D. degree from the University of Madras (Full time and Part time)

Sl.No.	Name of the Candidate	Title of the Ph.D. thesis	Part / Full time	Supervisor /Guide
1.	Mr. M. Radhakrishnan	Anti-TB drugs from actinomycytes	Full time	Dr. Vanaja Kumar
2.	Ms. Neema Boruai	Penicillin binding protein from M. tuberculosis & M.smegmatis	Full time	Dr. Sujatha Narayanan
3.	Mr.P. Dinesh Kumar	A molecular approach to the role of serine/ threonine kinase PknE in signal transduction involved in host pathogen interactions	Full time	Dr. Sujatha Narayanan
4.	Ms. Suba S.	Characterization of Lipoproteins of M.tb	Full time	Dr.Sujatha Narayanan
5.	Mr. Sameer Hassan	Genome analysis of phages and viruses	Full time	Dr. Vanaja Kumar
6.	Ms.R. Lakshmi	Molecular studies on mycobacteria	Full time	Dr. Vanaja Kumar
7.	Ms.N. Yamuna	Classification and regression trees	Full time	Dr.P. Venkatesan
8.	Mr. Jagadish Chandra Bose	Immunodominant epitopes against HIV subtype C	Part-time	Dr. Luke Elizabeth Hanna
9.	Mr.N. Arunkumar	Causal analysis	Part time	Dr.P. Venkatesan

List of students who have registered (full-time) for their Ph.D. programme with the University of Madras

Sl.No.	Name of theCandidate	Source of Funding	Title of the Ph.D. thesis	Supervisor/Guide
1.	Mr. Pugazhvendhan P.	ICMR	Immunoproteomic identification of B-cell antigens of M. tuberculosis	Dr. Alamelu Raja
2.	Ms.D. Santhi	ICMR-TASK FORCE	Novel subunit vaccine targets from M.tuberculosis	Dr. Alamelu Raja
3.	Ms.Maddineni Prabhavathi	CSIR	Immunoproteomically identified Mycobacterium tuberculosis antigens for diagnoosis	Dr. Alamelu Raja
4.	Mr.P. Balaji	ICMR	Diagnostic evaluation of novel T-cell antigens (Rv2204c, Rv2394) of M. tb	Dr. Alamelu Raja
5.	Ms.G. Akilandeswari	INSPIRE FELLOW	Structural characterization of three essential genes from M. tb	Dr. Alamelu Raja
6.	Ms.V. Malini	ICMR	Functional characterization of FtsY, a signal recognition particle receptor from M. tb	Dr. Sujatha Narayanan
7.	Mr. Srinivasan K.	NIH	Comparative genomics and pathogenesis of TB	Dr.Sujatha Narayanan
8.	Ms. Ahmed Kabir Refaya	ICMR	Mycobacterial transcriptional regulators in pathogenesis	Dr. Sujatha Narayanan
9.	M.V. Arunkumar	Lady Tata Memorial Trust	Gene regulation of mycobacteria	Dr. Sujatha Narayanan
10.	Ms.S. Priyadharshini	INSPIRE Fellow	Functional genomics of Mycobacterium tuberculosis	Dr. Sujatha Narayanan
11.	Mr. Brijendra Singh	CSIR	Chemokine gene polymorphism and chemokine expression in PTB	Dr.P. Selvaraj
12.	Mr. Afsal K.	ICMR	Effect of vitamin D3 on innate and adaptive immunity in pulmonary TB	Dr.P. Selvaraj
13.	Ms. Nancy Hilda J.	UGC	Neutrophil mediated innate immune response in TB	Dr.D. Sulochana
14.	Mr. Pawan Kumar N.	ICER	Pediatric TB	Dr. Luke Elizabeth Hanna
15.	Ms. Anuradha R.	ICER	Role of TLR in filarial pathology	Dr. Luke Elizabeth Hanna
16.	Mr. Narayanaiah Cheedarla	UGC	Comparative studies between HIV-1 and HIV-2 cases in India	Dr. Luke Elizabeth Hanna
17.	Mr. Jovvian George	ICER	Helminth Immunology	Dr. Luke Elizabeth Hanna
18.	Ms. Vidya Vijayan	INSPIRE FELLOW	Phage based drug target identification and anti-mycobacterial drug discovery	Dr. Luke Elizabeth Hanna
19.	Ms. A.S. Shainaba	UGC	Phage based drug target identification and anti-mycobacterial drug discovery	Dr. Vanaja Kumar

Staff registered (part-time) for their Ph.D. programme with the University of Madras, Chennai

Sl.No.	Name of the staff	Title of the Ph.D. thesis	Supervisor/Guide
1.	Mr. Anbalagan S.	Innate & adaptive immunity in HIV	Dr. Luke Elizabeth Hanna
2.	Mr. Harishankar M.	Role of vitamin D receptor promoter & 3’UTR gene variants on vitamin D modulated immune functions in TB	Dr.P.Selvaraj
3.	Mr. Sekar L.	Survival analysis	Dr.P. Venkatesan
4.	Mr. Sivakumar S.	Molecular epidemiology of TB	Dr. Sujatha Narayanan
5.	Mr. Srinivasan R.	Spatial analysis	Dr.P. Venkatesan
6.	Mr. Sukumar B.*	Statistical methods for micro array data analysis	Dr.P. Venkatesan
7.	Ms. Vasantha M.	Structural equation modeling	Dr.P. Venkatesan

* Ex-staff

STAFF LIST
(As on 1 April, 2013)

SCIENTIST ‘G’
1. Dr.Soumya Swaminathan, M.D., DNB

SCIENTIST ‘F’
1. Dr.Alamelu Raja, Ph.D.,
2. Dr.Vanaja Kumar, Ph.D.,
3. Dr.Sujatha Narayanan, Ph.D., CT.,

SCIENTIST ‘E’
1. Dr.K.Rajaram, M.B.B.S., DTRD
2. Dr.P.Selvaraj, Ph.D.,
3. Dr.P.Venkatesan, MPS, Ph.D., PGCDM, DSQCOR (ISI), SDS (ISI)
4. Dr.D.Sulochana, Ph.D.,
5. Dr.C.PadmaPriyadarsini, M.B.B.S.,DNB.,

SCIENTIST ‘D’
1. Dr.P.Paul Kumaran, M.B.B.S., M.P.H.,
2. Dr.D.Baskaran, M.B.B.S.,
3. Dr.Pradeep Aravindan Menon,MBBS,DPM
4. Dr.Sudha Subramanian, Ph.D.,

SCIENTIST ‘C’
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